# Thermo Scientific
# Avidin-Biotin Technical Handbook

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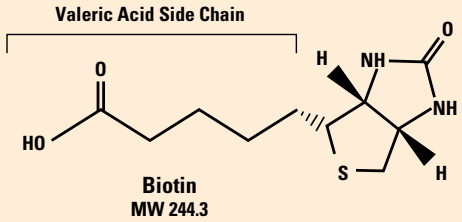
# Thermo Scientific EZ-Link Biotinylation Reagents

The highly specific interaction of avidin with biotin (vitamin H) can be a useful tool in designing nonradioactive purification and detection systems. The extraordinary affinity of avidin for biotin ($K_a = 10^{15}\ M^{-1}$) is the strongest known non-covalent interaction of a protein and ligand and allows biotin-containing molecules in a complex mixture to be discretely bound with avidin conjugates. Our extensive line of biotinylation reagents, conjugates and affinity supports exploits this unique interaction. Some applications in which the avidin-biotin interaction has been used include ELISA; immunohistochemical staining; Western, Northern and Southern blotting; immunoprecipitation; cell-surface labeling; affinity purification; and fluorescence-activated cell sorting (FACS).

Biotin, a 244 dalton vitamin found in tiny amounts in all living cells, binds with high affinity to avidin, streptavidin and Thermo Scientific NeutrAvidin Biotin-Binding Protein. Since biotin is a relatively small molecule, it can be conjugated to many proteins without significantly altering their biological activity. A protein can be reacted with several molecules of biotin that, in turn, can each bind a molecule of avidin. This greatly increases the sensitivity of many assay procedures.




The valeric acid side chain of the biotin molecule can be derivatized to incorporate various reactive groups that are used to attach biotin to other molecules. Using these reactive groups, biotin can be easily attached to most proteins and other molecules. Biotinylation reagents are available for targeting a variety of functional groups, including primary amines, sulfhydryls, carbohydrates and carboxyls. Photo-reactive biotin compounds that react nonspecifically upon photoactivation are also available. This variety of functional group specificities is extremely useful, allowing the choice of a biotinylation reagent that does not inactivate the target macromolecule. Several cleavable or reversible biotinylation reagents are also available and allow specific elution of the biotinylated molecule from biotin-binding proteins. A complete selection guide and detailed instructions for each reagent is available on the "Products" section of our web site.

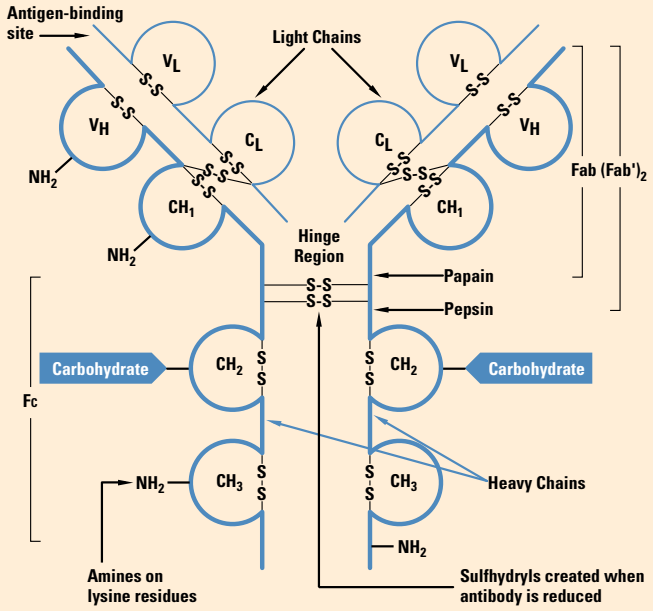
The most frequently used biotinylation reagents, *N*-hydroxysuccinimide (NHS) esters and *N*-hydroxysulfosuccinimide (sulfo-NHS) esters, react with primary amines. The functional groups available on the surface of the protein to be biotinylated may not be known. However, with most proteins, it is safe to assume that primary amines are available and accessible for biotinylation. The likelihood that primary amines are present increases as molecular weight increases. For example, BSA contains 59 primary amines and 30-35 of these are on the surface and can be reacted with NHS-esters.

While NHS-esters of biotin are the most frequently used biotinylation reagents, they are not necessarily the best for a particular application. If only a portion of the primary amines on a protein are reacted, reaction with NHS-esters of biotin will result in a random distribution of biotin on the surface of the protein. If a particular primary amine is critical to the biological activity of the protein, modification of this critical amine may result in the loss of its biological activity. Depending on the extent of biotinylation, complete loss of activity may occur.

Antibodies are biotinylated more often than any other class of proteins and it is advantageous to biotinylate in a manner that will maintain immunological reactivity. Thermo Scientific Sulfo-NHS-LC-Biotin is the number one choice for labeling both monoclonal and polyclonal antibodies because it is the simplest and often most effective method. The fast and reliable procedure has been optimized for antibody modification. If the antibody contains a lysine-rich antigen-binding site, amine-reactive reagents may inhibit antigen binding. One solution is to use biotin derivatives that react with sulfhydryl groups. By reducing the immunoglobulin under mild conditions, biotinylation can be isolated to free sulfhydryls generated from the hinge region. Another solution is to use a biotin derivative such as Biocytin-Hydrazide that reacts with aldehydes. Aldehydes can be generated on antibodies and other glycoproteins by oxidation of carbohydrates with periodate. Because carbohydrate is found selectively on the Fc portion of antibodies, biotin labeling is restricted from occurring near the antigen-binding site. This site-specific labeling method provides an antibody conjugate with the highest possible specific activity that is particularly important when antibody supply is limited and making the best possible use of the antibody is desired. This method is particularly useful for labeling polyclonal antibodies, which are heavily glycosylated.

Understanding the functional groups available on an antibody is the key to determining a strategy for modification.

- Primary amine groups ($-NH_2$) are found on lysine side chains and at the amino terminus of each polypeptide chain.
- Sulfhydryl groups (–SH) can be generated by reducing disulfide bonds in the hinge region.
- Carbohydrate residues containing cis-diols can be oxidized to create active aldehydes (–CHO).



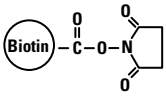
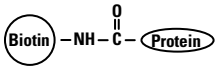
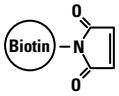
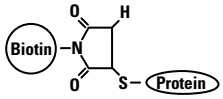
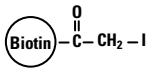
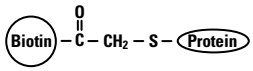
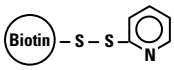
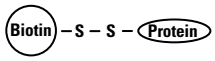
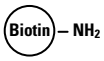
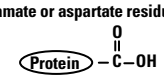
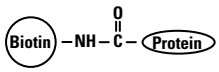
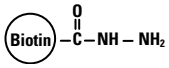
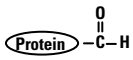
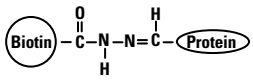
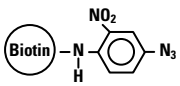
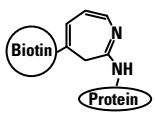
# Thermo Scientific Pierce Biotin-Labeling Reagent Selection Guides

These selection guides are designed to help you quickly choose the most appropriate biotinylation reagent to use for an application. Selection Guide 1 focuses on the purpose/application of biotin labeling and is organized by the type of molecule that is to be labeled. It includes only the most common biotinylation reagent choices. Selection Guide 2 focuses on reaction chemistries and is organized according to the functional groups present on the molecule to be labeled. For a more complete selection guide and for detailed product instructions, visit the "Products" section of our web site.

## Selection Guide 1

| Biotinylation Target | Application | Thermo Scientific Pierce Reagent(s) | Features/Benefits |
|---|---|---|---|
| **Antibody** | ELISA or blotting (to detect with streptavidin-HRP) or affinity-purifying antigen (after immobilizing on streptavidin column) | Sulfo-NHS-LC-Biotin (Product # 21335, Kit Product # 21435, 21935) | Amine-reactive (lysines). Sure to work well for nearly any antibody. Very simple and direct. |
| | | NHS-$PEG_4$-Biotin (Product # 21329, 21330, 21362) | Amine-reactive (lysines). PEG spacer arm increases solubility of biotinylated molecule. |
| | | Biotin-$PEG_4$-Hydrazide (Product # 21360), plus Sodium meta-Periodate (Product # 20504) | Carbohydrate-reactive. Primarily for polyclonal antibodies (purified from serum), which usually have required carbohydrate side chains. |
| **Purified protein** | ELISA or blotting (to detect with streptavidin-HRP) or affinity-purifying receptor or antibody (after immobilizing on streptavidin column) | Sulfo-NHS-LC-Biotin (Product # 21335, Kit Product # 21435, 21935, 21362) | Amine-reactive (lysines). Obvious choice for sizeable proteins. Slight risk of blocking epitopes or binding sites necessary for other purposes. |
| | | NHS-$PEG_4$-Biotin (Product # 21329, 21330) | Amine-reactive (lysines). PEG spacer arm increases solubility of biotinylated molecule. |
| | | Sulfo-NHS-SS-Biotin (Product # 21331) | Amine-reactive (lysines). Cleavable disulfide spacer allows recovery of biotinylated protein from immobilized streptavidin. |
| | | Maleimide-$PEG_2$-Biotin (Product # 21901, 21902) | Sulfhydryl-reactive. Use only when there is a reason to target sulfhydryls rather than amines. |
| | | Biotin-$PEG_4$-Hydrazide (Product # 21360), plus Sodium Periodate (Product # 20504) | Carbohydrate-reactive. Good choice if the protein is known to be adequately glycosylated and the carbohydrate is not important for downstream applications. |
| **Purified peptide** | ELISA or blotting (to detect with streptavidin-HRP) or affinity-purifying receptor or antibody (after immobilizing on streptavidin column) | Sulfo-NHS-LC-Biotin (Product # 21335, Kit Product # 21435, 21935) | Amine-reactive. Choose if *N*-terminus or lysines are not required for binding reactions. May be difficult to remove unreacted reagent. |
| | | NHS-$PEG_4$-Biotin (Product # 21329, 21330) | Amine-reactive (lysines). PEG spacer arm increases solubility of biotinylated molecule. |
| | | Maleimide-$PEG_2$-Biotin (Product # 21901, 21902), plus Immobilized TCEP (Product # 77712) | Sulfhydryl-reactive. Choose if cysteines are not required for binding reactions (often a terminal cysteine is added during peptide synthesis for this purpose). Must ensure that peptide is reduced. May be difficult to remove unreacted reagent. |
| | | Amine-$PEG_2$-Biotin (Product # 21346), plus EDC (Product # 22980) and Sulfo-NHS (Product # 24510) | Carbohydrate and amine-reactive. Use only if some peptide polymerization is acceptable along with biotinylation. Polymerization may be desirable if it creates large enough molecules to recover conjugate by dialysis. |
| **Cell surface proteins** | Primarily for affinity-purifying or removing cell surface receptor ligands (after immobilizing on streptavidin column) | Sulfo-NHS-LC-Biotin (Product # 21335, Kit Product # 21435, 21935) | Amine-reactive (lysines). Most commonly-used and most general choice. |
| | | NHS-$PEG_4$-Biotin (Product # 21329, 21330) | Amine-reactive (lysines). PEG spacer arm increases solubility of biotinylated molecule. |
| | | Sulfo-NHS-SS-Biotin (Product # 21331) | Amine-reactive (lysines). Cleavable disulfide spacer allows recovery of biotinylated protein from immobilized NeutrAvidin Protein. |
| | | Cell Surface Protein Isolation Kit (Product # 89888) | Amine-reactive (lysines). Cleavable disulfide spacer allows recovery of biotinylated protein from immobilized NeutrAvidin™ Protein. |
| **DNA/RNA or oligo-nucleotides** | Capture or detection of oligonucleotides in ELISA-type applications or affinity purification (Capture in streptavidin-coated plates or detect protein-bound oligo with streptavidin-HRP) | Psoralen-$PEG_3$-Biotin (Product # 29986) | Provides random labeling of nucleic acid backbone. May cause steric hindrance for hybridization. methods. |
| | | Amine-$PEG_2$-Biotin (Product # 21346), plus EDC (Product # 22980) and Sulfo-NHS (Product # 24510) | Requires intact 5'-phosphate; produces end-labeled oligo. |

## Selection Guide 2

| Reactive Group | Reacts With | Linkage Formed |
|---|---|---|
| **NHS-Ester/Sulfo-NHS Ester**  | **Primary Amine (lysine residue)**  | **Amide Bond**  |
| **Maleimide**  | **Sulfhydryl (cysteine residue – not disulfide bonded)**  | **Thioether Bond**  |
| **Iodoacetyl**  | | **Thioether Bond**  |
| **Pyridyl Disulfide**  | | **Disulfide Bond**  |
| **Amine**  | **Carboxyl** (glutamate or aspartate residues)  (Reaction requires EDC cross-linker) | **Amide Bond**  |
| **Hydrazide**  | **Oxidized Carbohydrate**  | **Hydrazone Bond**  |
| **Azido (Photoactivatable)**  | **DNA/RNA, Protein, Carbohydrates** |  Ring expansion followed by coupling with primary amine or insertion into double bonds |

## Biotinylation Reagents

*Label almost anything with biotin using one of our many high-quality biotinylation reagents*

Whether you are an expert or are trying biotinylation for the first time, Thermo Scientific EZ-Link Reagents and Kits make biotinylation easy. Our customers depend on our consistent lot-to-lot quality for reproducible results. We offer complete EZ-Link® Biotinylation Kits with buffers, fast and efficient spin desalting columns and HABA detection reagent to determine the number of biotin residues attached to your protein.

### Amine-Reactive Biotinylation Reagents

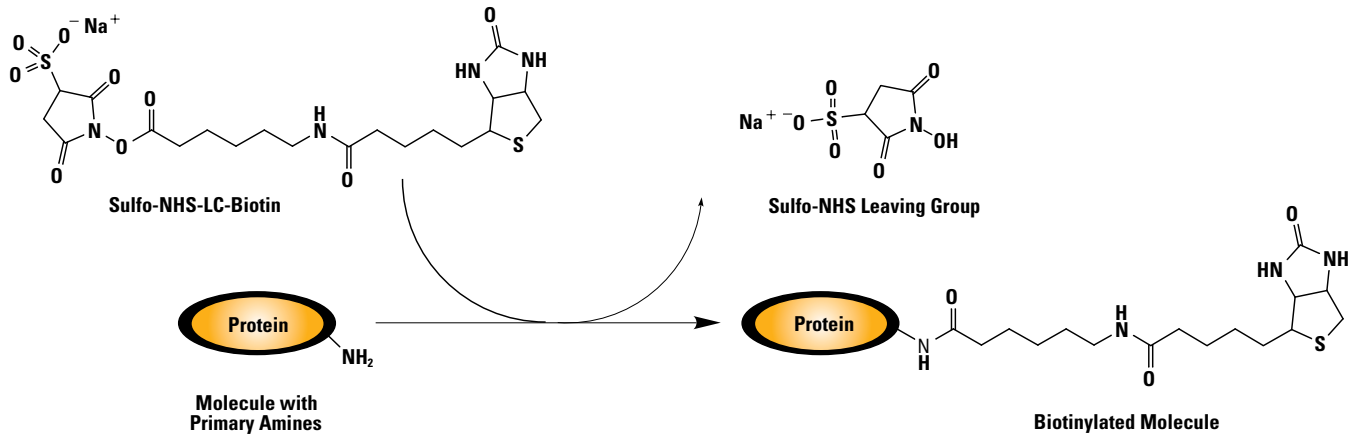
The most common target for modifying protein molecules is the amine group, which is present on the vast majority of proteins due to the abundance of lysine side chain ε-amines and *N*-terminal α-amines. Based on water solubility, amine-reactive biotinylation reagents can be divided into two groups. NHS-esters of biotin are essentially water-insoluble. For reactions in aqueous solution, they must first be dissolved in an organic solvent, then diluted into the aqueous reaction mixture. The most commonly used organic solvents for this purpose are DMSO and DMF, which are compatible with most proteins at 20% final concentration. The solvent acts as a carrier for the biotinylation reagent, forming a microemulsion in the aqueous phase and allowing the biotinylation reaction to proceed. The water-insoluble NHS-esters of biotin are membrane-permeable because they do not possess a charged group. They may be used for biotinylating internal as well as external components of a cell.

Sulfo-NHS-esters of biotin are soluble up to approximately 10 mM in water. Sulfo-NHS-esters should be dissolved in water just before use because they are prone to hydrolysis. The water solubility of sulfo-NHS-esters results from the presence of the sulfonate ($-SO_3$) group on the *N*-hydroxysuccinimide ring and eliminates the need to dissolve the reagent in an organic solvent. These compounds are used for applications that cannot tolerate organic solvents. Sulfo-NHS-esters of biotin are also recommended for use as cell surface biotinylation reagents. Because of the charged sulfonate group, these compounds do not penetrate the plasma membrane; thus biotinylation is restricted to the cell surface.

The reaction chemistries of NHS- and sulfo-NHS-esters are essentially identical: an amide bond is formed and NHS or sulfo-NHS are leaving groups in the reaction. Because the target for the ester is the deprotonated form of the primary amine, the reaction becomes significant at neutral pH values and above when the amine is able to react with the ester by nucleophilic attack. Hydrolysis of the NHS-ester is a major competing reaction, and the rate of hydrolysis increases with increasing pH. NHS- and sulfo-NHS-esters have a half-life of hydrolysis of 2-4 hours at pH 7. This half-life decreases to just a few minutes at pH 9.

There is considerable flexibility in the actual conditions used for conjugating NHS-esters (or sulfo-NHS-esters) to primary amines. Incubation temperatures range from 4-37°C, reaction mixture pH values range from 7-9, and incubation times range from a few minutes to overnight. Buffers containing amines (such as Tris or glycine) must be avoided because they compete with the reaction. In preparing an NHS-ester biotin conjugate, a particular set of conditions will result in a conjugate with optimum properties for a specific application. The preparation of an optimum conjugate is largely dependent on the degree of incorporation of the label. Because of the variability among proteins, especially the number of amines available for conjugation, conjugation conditions that are optimal for one protein may not be optimal for another protein.

There are several additional features to consider in an amine-reactive biotinylation reagent. Because biotin binds in a pocket located 9 Å below the surface of the avidin molecule, the spacer arm connected to the biotin is critical. Long spacer arms reduce steric hindrance and result in enhanced interaction of avidin and biotin. It is sometimes necessary to remove biotin from a molecule once a procedure has been completed, and there are reagents with a cleavable spacer arm that allow this procedure. A macromolecule is first reacted with the cleavable biotinylation reagent, then it is used in a detection or purification system. Finally the biotin moiety can be cleaved away, releasing the molecule into solution. Biotin labeling generally reduces the solubility of a molecule and may result in precipitation. Unlike sulfo-NHS-esters that lose their solubility-enhancing sulfonate group during the reaction process, reagents containing a PEG spacer arm retain their high solubility when bound to a protein and are an ideal choice when precipitation must be overcome.




**Reaction of Sulfo-NHS-LC-Biotin with a primary amine.**

## Thermo Scientific EZ-Link Amine-Reactive Biotinylation Kits

| Kit | Highlights | References | Ordering Information | | |
|---|---|---|---|---|---|
| | | | Product # | Description | Pkg. Size |
| **Sulfo-NHS Biotinylation Kits** | • Complete kit with an optimized protocol for labeling a protein and determining how much biotin has been attached<br>• Fast and efficient labeling procedure | 1, 2 | **21425** | **EZ-Link Sulfo-NHS Biotinylation Kit**<br>Includes: Sulfo-NHS-Biotin<br>Reaction Buffer<br>Zeba™ Spin Columns<br>HABA Dye (10 mM)<br>Avidin, affinity-purified | Kit<br>25 mg<br>1 pack (500 ml)<br>10<br>1 ml<br>10 mg |
| | | | **21925** | **EZ-Link Micro Sulfo-NHS Biotinylation Kit**<br>Includes: No-Weigh™ Sulfo-NHS-Biotin<br>Zeba Desalting Columns<br>BupH™ Phosphate Buffered Saline | Kit<br>8 x 1 mg<br>2 ml<br>1 pack (500 ml) |
| **Sulfo-NHS-LC Biotinylation Kits** | • Complete kit with an optimized protocol for labeling a protein and determining how much biotin has been attached<br>• Fast and efficient labeling procedure | 1, 2 | **21435** | **EZ-Link Sulfo-NHS-LC Biotinylation Kits**<br>Includes: Sulfo-NHS-LC-Biotin<br>Reaction Buffer<br>Zeba Spin Columns<br>HABA Dye (10 mM)<br>Avidin, affinity-purified | Kit<br>25 mg<br>1 pack (500 ml)<br>10<br>1 ml<br>10 mg |
| | | | **21935** | **EZ-Link Micro Sulfo-NHS-LC Biotinylation Kit**<br>Includes: No-Weigh Sulfo-NHS-LC Biotin<br>Zeba Desalting Columns<br>BupH Phosphate Buffered Saline | Kit<br>8 x 1 mg<br>2 ml<br>1 pack (500 ml) |
| **NHS-PEG$_4$ Biotinylation Kits** | • PEG$_4$-Biotin transfers water-solubility to biotinylated molecules | 3-6 | **21455** | **EZ-Link NHS-PEG$_4$ Biotinylation Kit**<br>Includes: NHS-PEG$_4$-Biotin<br>BupH PBS<br>Zeba Spin Columns<br>HABA Dye (10 mM)<br>Avidin, a ffinity-purified | Kit<br>8 x 2 mg<br>1 pack (500 ml)<br>10<br>1 ml<br>10 mg |
| | | | **21955** | **EZ-Link Micro NHS-PEG$_4$ Biotinylation Kit**<br>Includes: No-Weigh NHS-PEG$_4$-Biotin<br>Zeba Desalting Columns<br>BupH Phosphate Buffered Saline | Kit<br>8 x 1 mg<br>2 ml<br>1 pack (500 ml) |
| **Sulfo-NHS-SS Biotinylation Kits** | • Cleavable biotinylation reagent allows removal of biotin label | 7-9 | **21445** | **EZ-Link Sulfo-NHS-SS Biotinylation Kit**<br>Includes: Sulfo-NHS-SS-Biotin<br>Non-reagent contents same as Product # 21425 | Kit<br>25 mg |
| | | | **21945** | **EZ-Link Micro Sulfo-NHS-SS Biotinylation Kit**<br>Includes: Sulfo-NHS-SS-Biotin<br>Non-reagent contents same as Product # 21925 | Kit<br>8 x 1 mg |


**References**

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3. Ali, M.K. and Bergson, C. (2003). *J. Biol. Chem.* **278**, 51654-51663.
4. Du, J., *et al.* (2003). *J. Cell Biol.* **163(2)**, 385-395.
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7. Daniels, G.M. and Amara, S.G. (1998). *Methods. Enzymol.* **296**, 307-318.
8. Huh, K-H. and Wenthold, R.J. (1999). *J. Biol. Chem.* **274**, 151-157.
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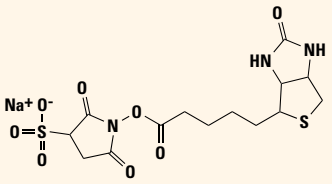
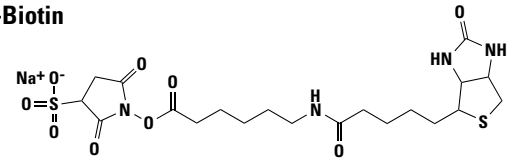
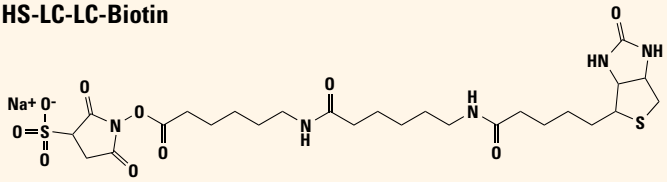
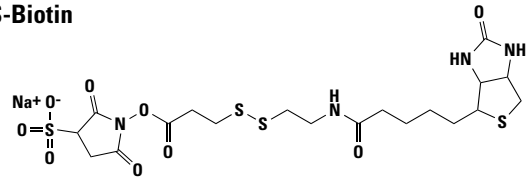
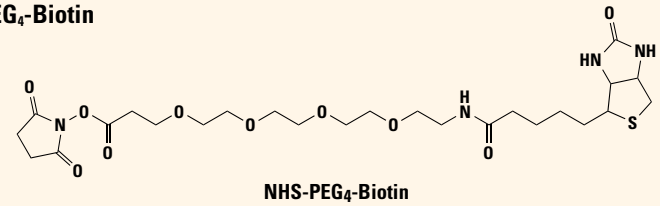
# Amine-Reactive Biotinylation Reagents

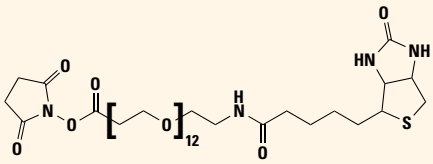
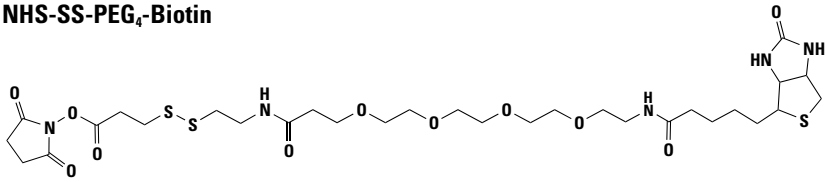
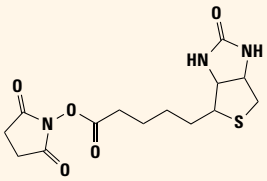
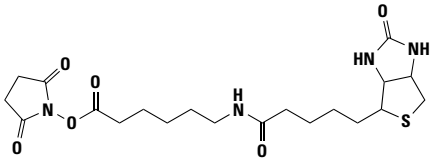
## Thermo Scientific EZ-Link Amine-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Sulfo-NHS-Biotin** |  **Sulfo-NHS-Biotin** MW 443.43 Spacer Arm 13.5 Å | • Reacts with primary amines at ph 7-9 in non-amine-containing buffers<br>• Water-soluble for ease of use |
| **Sulfo-NHS-LC-Biotin** |  **Sulfo-NHS-LC-Biotin** MW 556.59 Spacer Arm 22.4 Å | • Water-soluble for ease of use<br>• Ideal labeling reagent for monoclonal and polyclonal antibodies |
| **Sulfo-NHS-LC-LC-Biotin** |  **Sulfo-NHS-LC-LC-Biotin** MW 669.75 Spacer Arm 30.5 Å | • Contains an extra-long spacer arm (30.5 Å) to reduce steric hindrance |
| **Sulfo-NHS-SS-Biotin** |  **Sulfo-NHS-SS-Biotin** MW 606.69 Spacer Arm 24.3 Å | • Disulfide linkage can be cleaved by reducing agents<br>• Ideal reagent for reversibly biotinylating cell surface proteins |
| **NHS-PEG$_4$-Biotin** |  **NHS-PEG$_4$-Biotin** MW 588.67 Spacer Arm 29.0 Å | • PEG spacer arm increases solubility of conjugates<br>• No-Weigh Packaging ensures fresh reagent and eliminates tedious weighing of reagent |

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Arosa, F.A., *et al.* (1999). *J. Biol. Chem.* **274**, 16917-16922.<br>• Claypool, S.M., *et al.* (2002). *J. Biol. Chem.* **277**, 28038-28050.<br>• Ellerbroek, S.M., *et al.* (2001). *J. Biol. Chem.* **276**, 24833-24842.<br>• Leighton, B.H., *et al.* (2002). *J. Biol. Chem.* **277**, 29847-29855.<br>• Neely, K.E., *et al.* (2002). *Mol. Cell. Biol.* **22**, 1615-1625. | **21217** | **EZ-Link Sulfo-NHS-Biotin** | 50 mg |
| | **21326** | **No-Weigh Sulfo-NHS-Biotin** | 8 x 1 mg |
| • Arosa, F.A., *et al.* (1999). *J. Biol. Chem.* **274**, 16917-16922.<br>• Baqui, M., *et al.* (2003). *J. Biol. Chem.* **278**, 1206-1211.<br>• Huh, K-H. and Wenthold, R.J. (1999). *J. Biol. Chem.* **274**, 151-157.<br>• Lesa, G.M., *et al.* (2000). *J. Biol. Chem.* **275**, 2831-2836.<br>• Liaw, P.C.Y., *et al.* (2001). *J. Biol Chem.* **276**, 8364-8370.<br>• Liu, L.A. and Engvall, E. (1999). *J. Biol. Chem.* **274**, 38171-38176. | **21335** | **EZ-Link Sulfo-NHS-LC-Biotin** | 100 mg |
| | **21327** | **No-Weigh Sulfo-NHS-LC-Biotin** | 8 x 1 mg |
| • Muroi, M., *et al.* (2002). *J. Biol. Chem.* **277**, 42372-42379.<br>• Schwarzman, A.L., *et al.* (1999). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7932-7937. | **21338** | **EZ-Link Sulfo-NHS-LC-LC-Biotin** | 50 mg |
| • Daniels, G.M. and Amara, S.G. (1998). *Methods. Enzymol.* **296**, 307-318.<br>• Huh, K-H. and Wenthold, R.J. (1999). *J. Biol. Chem.* **274**, 151-157.<br>• Trotti, D., *et al.* (2001). *J. Biol. Chem.* **276**, 576-582. | **21331** | **EZ-Link Sulfo-NHS-SS-Biotin** | 100 mg |
| | **21328** | **No-Weigh Sulfo-NHS-SS-Biotin** | 8 x 1 mg |
| • Dodeller, F., *et al.* (2008) *J. Biol. Chem.* **283**, 21487-21494.<br>• Newton, J.R., *et al.* (2007) *J. Nucl. Med.* **48**, 429-436.<br>• Behrens, M. *et al.* (2006) *J. Biol. Chem.* **281**, 20650-20659. | **21329** | **No-Weigh NHS-PEG$_4$-Biotin** | 8 x 2 mg |
| | **21330** | **EZ-Link NHS-PEG$_4$-Biotin** | 25 mg |
| | **21362** | **EZ-Link NHS-PEG$_4$-Biotin** | 50 mg |
| | **21363** | **EZ-Link NHS-PEG$_4$-Biotin** | 1 g |

# Amine-Reactive Biotinylation Reagents

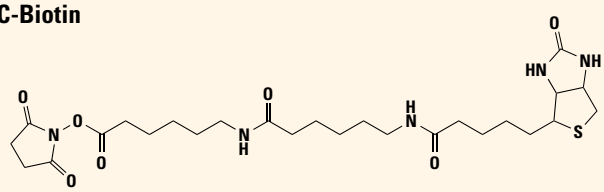
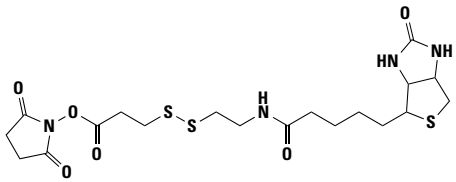
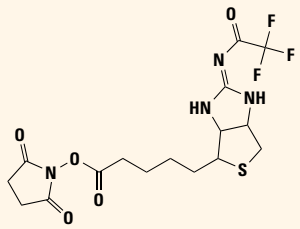
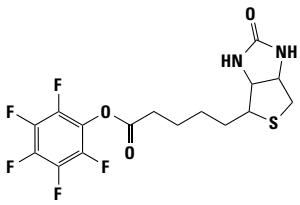
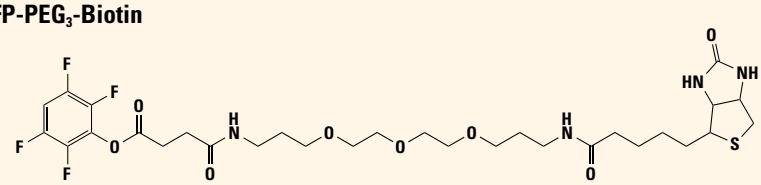
## Thermo Scientific EZ-Link Amine-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **NHS-PEG$_{12}$-Biotin** |  **NHS-PEG$_{12}$-Biotin** MW 941.09 Spacer Arm 56 Å | • PEG spacer arm increases solubility of conjugates<br>• Extra long spacer arm reduces steric hindrance |
| **NHS-SS-PEG$_4$-Biotin** |  **NHS-SS-PEG$_4$-Biotin** M.W. 751.94 Spacer Arm 37.9 Å | • PEG spacer arm increases solubility of conjugates<br>• Disulfide linkage can be cleaved by reducing agents |
| **NHS-Biotin** |  **NHS-Biotin** MW 341.38 Spacer Arm 13.5 Å | • Must be dissolved in DMSO or DMF before adding to aqueous solution<br>• Able to penetrate cell membranes |
| **NHS-LC-Biotin** |  **NHS-LC-Biotin** MW 454.54 Spacer Arm 22.4 Å | • Must be dissolved in DMSO or DMF before adding to aqueous solution<br>• Able to penetrate cell membranes |

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| | **21312** | **EZ-Link NHS-PEG$_{12}$-Biotin** | 25 mg |
| | **21313** | **EZ-Link NHS-PEG$_{12}$-Biotin** | 500 mg |
| | **21442** | **NHS-SS-PEG$_4$-Biotin** | 50 mg |
| • Fouassier, L., *et al.* (2000). *J. Biol. Chem.* **275**, 25039-25045.<br>• Nunomura, W., *et al.* (2000). *J. Biol. Chem.* **275**, 6360-6367. | **20217** | **EZ-Link NHS-Biotin** | 100 mg |
| • Chiu, N.H., *et al.* (1999). *Clin. Chem.* **45**, 1954-1959.<br>• Schumacher, T.N., *et al.* (1996). *Science* **271**, 1854-1857.<br>• Tang, A., *et al.* (1993). *Nature* **361**, 82-85. | **21336** | **EZ-Link NHS-LC-Biotin** | 50 mg |

# Amine-Reactive Biotinylation Reagents

## Thermo Scientific EZ-Link Amine-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **NHS-LC-LC-Biotin** |  **NHS-LC-LC-Biotin** MW 567.70 Spacer Arm 30.5 Å | • Contains an extra-long spacer arm (30.5 Å) to reduce steric hindrance |
| **NHS-SS-Biotin** |  **NHS-SS-Biotin** MW 504.65 Spacer Arm 24.3 Å | • Label almost any protein with biotin to facilitate immobilization, purification or detection<br>• Disulfide bond in the spacer arm allows the biotin label to be removed<br>• Amine-reactive NHS ester reacts rapidly with any primary amine-containing molecule to attach the biotin via a stable amide bond<br>• Long spacer arm on the biotin reduces steric hindrance with binding to avidin or other biotin-binding proteins |
| **NHS-Iminobiotin** |  **NHS-Iminobiotin Trifluoroacetamide** MW 436.41 Spacer Arm 13.5 Å | • Reversible binding to avidin prevents protein denaturation during purification<br>• Binds to avidin at pH >9.5 and dissociates at pH 4 |
| **PFP-Biotin** |  **Biotin-PFP Ester** MW 410.36 Spacer Arm 9.6 Å | • More reactive than NHS esters<br>• Will react with primary and secondary amines at pH 7-9<br>• Easier-to-handle and store than NHS esters<br>• Great for DNA labeling<br>• PEG spacer arm increases solubility of conjugates |
| **TFP-PEG$_3$-Biotin** |  **TFP-PEG$_3$-Biotin** MW 694.74 Spacer Arm 32.6 Å | • Long-chain, water-soluble, polyethylene oxide (PEG) spacer arm<br>• Tetrafluorophenyl (TFP) ester reacts with primary and secondary amines at pH 7-9<br>• Glycine and Tris buffers interfere with reaction<br>• PEG spacer arm transfers hydrophilicity to final conjugate |

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Bradley, C., *et al.* (2007) *Carcinogen.* **28**, 2184-2192.<br>• Sehr, P., *et al.* (2007) *J. Biomo.l Screen.* **12**, 560-567.<br>• Negishi, A., *et al.* (2004) *Glycobiolog.* **14**, 969-977. | **21343** | **EZ-Link NHS-LC-LC-Biotin** | 50 mg |
| | **21441** | **EZ-Link NHS-SS-Biotin** | 50 mg |
| • Orr, G.A., *et al.* (1981). *J. Biol. Chem.* **256**, 761-766.<br>• Zeheb, R., *et al.* (1983). *Anal. Biochem.* **129**, 156-161. | **21117** | **EZ-Link NHS-Iminobiotin** | 100 mg |
| • Muroi, M., *et al.* (2002). *J. Biol. Chem.* **277**, 42372-42379.<br>• Michaelis, K., *et al.* (2006) *J. Pharmacol. Exp. Ther.* **317**, 1246-1253. | **21218** | **EZ-Link PFP-Biotin** | 50 mg |
| | **21219** | **EZ-Link TFP-PEG$_3$-Biotin** | 50 mg |

# Sulfhydryl-Reactive Biotinylation Reagents

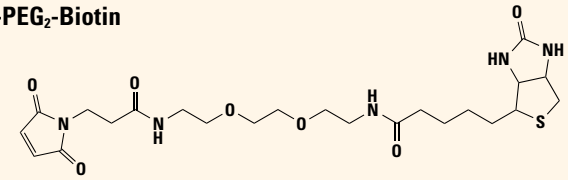
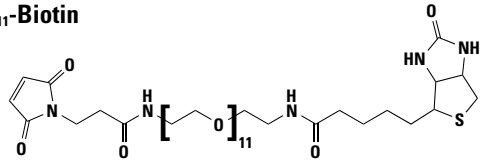
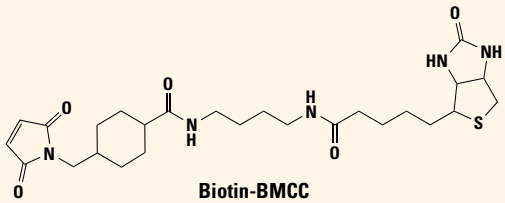
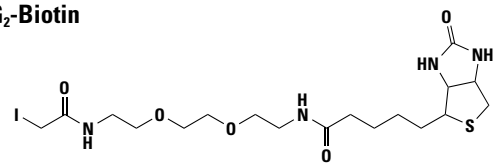
## Sulfhydryl-Reactive Biotinylation Reagents

The second most common target for modification in biological molecules is free sulfhydryl groups, which are found in the form of exposed cysteine in a protein or peptide. Sulfhydryl group biotinylation may provide an advantage in some applications; for example, targeting a sulfhydryl group can be used as a method for preserving the biological activity of an enzyme when amines are found at the active site. Modification of these amines may render the enzyme inactive. This complication can be avoided by using derivatives of biotin that react with sulfhydryls. Because biotinylation with these reagents must be performed in buffers free of extraneous sulfhydryls, substances such as 2-mercaptoethanol, dithiothreitol and mercaptoethylamine must be removed before biotinylation.

Proteins, peptides or other molecules to be biotinylated by sulfhydryl-reactive reagents must have a free sulfhydryl group (–SH) available; disulfides will not react with sulfhydryl-specific biotinylation reagents. If free sulfhydryls are not available, they can be generated from disulfides by incubation with a reducing agent or from lysine residues by incubating with modification reagents such as 2-Iminothiolane (Traut's Reagent) or SATA. When working with free sulfhydryls, EDTA is generally included in the buffer system for its antioxidative effect. EDTA chelates trace amounts of metals in solution that promote disulfide bond formation. Using nitrogen-purged buffers is an additional precaution to prevent oxidation of the free sulfhydryls.

Three separate reaction chemistries are employed to target sulfhydryl groups for biotinylation. The most specific method uses reactive maleimide groups, which are 1,000 times more reactive toward free sulfhydryls than toward amines at pH 7. Biotin-BMCC must be dissolved in an organic solvent, then diluted into an aqueous reaction mixture. Maleimide-$PEG_2$-Biotin, which is water-soluble by virtue of its polyethyleneoxide (PEG) spacer arm, may be dissolved directly in aqueous solution. The reaction of maleimide with free thiols is carried out at pH 6.5-7.5 because cross-reactivity

## Thermo Scientific EZ-Link Sulfhydryl-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Maleimide-$PEG_2$-Biotin** |  **Maleimide-$PEG_2$-Biotin** MW 525.62 Spacer Arm 29.1 Å | • Water-soluble<br>• Reacts with sulfhydryl (–SH) groups at acidic to neutral pH<br>• Avoids potential modification of tyrosine residues that can be associated with Iodoacetyl-Biotin |
| **Maleimide-$PEG_{11}$-Biotin** |  **Maleimide-$PEG_{11}$-Biotin** MW 922.09 Spacer Arm 59.1 Å | • Water-soluble<br>• Reacts with sulfhydryl (-SH) groups at acidic to neutral pH<br>• Extra long spacer arm reduces steric hindrance |
| **Biotin-BMCC** |  **Biotin-BMCC** MW 533.68 Spacer Arm 32.6 Å | • Reacts with sulfhydryl (–SH) groups at pH 6.5-7.5<br>• Avoids potential modification of tyrosine residues that can be associated with Iodoacetyl-Biotin<br>• Must be dissolved in DMSO or DMF before use |
| **Iodoacetyl-$PEG_2$-Biotin** |  **Iodoacetyl-$PEG_2$-Biotin** MW 542.43 Spacer Arm 24.7 Å | • Water-soluble<br>• Reacts with sulfhydryl (–SH) groups at basic to neutral pH<br>• Coupling reactions occur in the dark |

toward primary amines can occur at higher pH values. Hydrolysis of the maleimide group also increases at higher pH values.

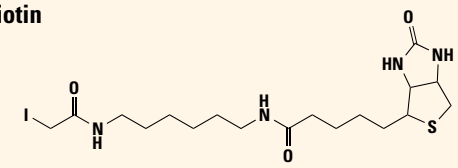
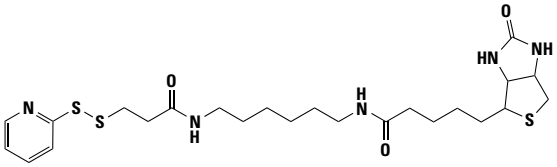
Iodoacetyl groups are also used to target free sulfhydryls. Iodoacetyl-LC-Biotin is not water-soluble and must be dissolved in a solvent before use in an aqueous reaction mixture. Iodoacetyl-$PEG_2$-Biotin, which is water-soluble by virtue of its PEG spacer arm, may be dissolved directly in aqueous solution. The iodoacetyl group reacts mainly with thiol groups at pH 7.5-8.5, resulting in a stable thioether bond. Unless precautions are taken, iodoacetyl groups may not be specific for sulfhydryls. The reaction can be directed toward sulfhydryl groups by limiting the molar ratio of Iodoacetyl-Biotin to protein, such that the concentration of biotin is present at a small excess over the sulfhydryl content. Also, the reaction pH should be maintained in the range of 7.5-8.5. Below pH 9, cross-reactivity with amine, thioether and imidazole groups is minimized. Therefore, maintaining a lower pH ensures the modification of sulfhydryl groups and not amino groups. If there are no cysteines available, the reaction can be directed at imidazoles by adjusting the pH to 6.9-7.0. However, the incubation time must be increased to a week. Histidyl side chains and amino groups react in the deprotonated form and may take part in reactions above pH 5 and pH 7, respectively, although this reaction is much slower than that for sulfhydryls.

A pyridyldithiol group may also be used to attach biotin to a free sulfhydryl by disulfide exchange, resulting in the formation of a mixed disulfide bond. The reaction of Biotin-HPDP is generally carried out under physiologic conditions although the process occurs within a wide range of pH conditions and with a variety of buffer components. Pyridyldithiol reactions result in the release of pyridine-2-thione, which cannot react with free sulfhydryls. The release of this compound can be measured and the reaction progress monitored by an increasing absorbance at 343 nm. Another important feature of this method is that the biotin is released by treatment with reducing agents.

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Oda, Y., *et al.* (2001). *Nat. Biotechnol.* **19,** 379-382.<br>• Inglis, K.J., *et al.* (2008) *J. Biol. Chem.* doi:10.1074/jbc.C800206200 | **21901** | **EZ-Link Maleimide-$PEG_2$-Biotin** | 50 mg |
| | **21902** | **No-Weigh Maleimide-$PEG_2$-Biotin Microtubes** | 8 x 2 mg |
| | **21911** | **Maleimide-$PEG_{11}$-Biotin** | 25 mg |
| • Roberts, P.J., *et al.* (2008) *J. Biol. Chem.* **283,** 25150-25163.<br>• Shi, M., *et al.* (2007) *J. Biol. Chem.* **282,** 30198-30206.<br>• Chenette, E. J., *et al.* (2006) *Mol. Biol. Cell.* **17,** 3108-3121. | **21900** | **EZ-Link Biotin-BMCC** | 50 mg |
| • Kim, K., *et al.* (2001). *J. Biol. Chem.* **276,** 40591-40598.<br>• Muroi, M., *et al.* (2002). *J. Biol. Chem.* **277,** 42372-42379. | **21334** | **EZ-Link Iodoacetyl-$PEG_2$-Biotin** | 50 mg |

# Sulfhydryl-Reactive Biotinylation Reagents

## Thermo Scientific EZ-Link Sulfhydryl-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Iodoacetyl-LC-Biotin** |  <br>**Iodoacetyl-LC-Biotin**<br>**MW 510.43**<br>**Spacer Arm 27.1 Å** | • Reacts with sulfhydryl (–SH) groups at basic to neutral pH<br>• Coupling reactions occur in the dark<br>• Must be dissolved in DMSO or DMF before use |
| **Biotin-HPDP** |  <br>**Biotin-HPDP**<br>**MW 539.78**<br>**Spacer Arm 29.2 Å** | • Pyridyldithiol reacts with sulfhydryl (–SH) groups to form a stable disulfide bond between pH 6-9<br>• Pyridine-2-thione leaving group can be used to measure degree of biotinylation at 343 nm<br>• Disulfide bond is cleavable using 50 mM DTT or 100 mM 2-mercaptoethanol<br>• Must be dissolved in DMSO or DMF before use |

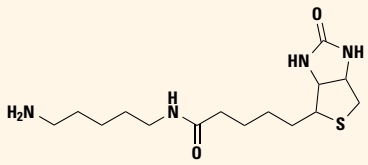
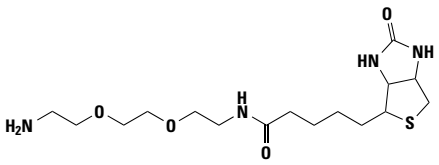
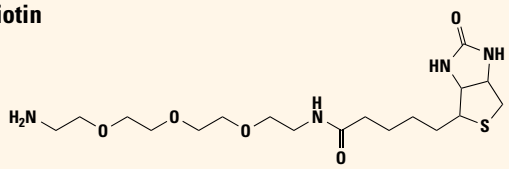
| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Sutoh, K., *et al.* (1984). *J. Mol. Biol.* **178,** 323-339.<br>• Yamamoto, K., *et al.* (1984). *FEBS Lett.* **176,** 75-78. | **21333** | **EZ-Link Iodoacetyl-LC-Biotin** | 50 mg |
| • Ishmael, F.T., *et al.* (2001). *J. Biol. Chem.* **276,** 25236-25242.<br>• Slatin, S.L., *et al.* (2002). *Proc. Natl. Acad. Sci. U.S.A.* **99,** 1286-1291. | **21341** | **EZ-Link Biotin-HPDP** | 50 mg |

# Carboxyl-Reactive Biotinylation Reagents

### Carboxyl-Reactive Biotinylation Reagents

Carboxyl groups, in the form of carboxy termini, aspartate residues or glutamate residues, can also be targeted for biotin labeling using amine-derivatized biotin molecules. This reaction is mediated by a class of crosslinkers known as carbodiimides and results in the formation of an amide bond. The reaction with EDC, the most common carbodiimide crosslinker, is generally performed in an MES buffer at pH 4.5-5 and requires just minutes to complete. Buffers containing primary amines (Tris, glycine, etc.) or carboxyls (acetate, citrate, etc.) must be avoided because they will quench the reaction. Phosphate buffers are also not recommended because they reduce the conjugation efficiency, although this effect can be overcome by adding more EDC.

## Thermo Scientific EZ-Link Carboxyl-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Pentylamine-Biotin** |  <br>**Pentylamine-Biotin**<br>**MW 328.47**<br>**Spacer Arm 18.9 Å** | • Use the EDC crosslinker to couple this analog to carboxyl (–COOH) groups<br>• Dissolves in aqueous solutions<br>• Coupling with EDC occurs at pH 4-6 |
| **Amine-PEG$_2$-Biotin** |  <br>**Amine-PEG$_2$-Biotin**<br>**MW 374.50**<br>**Spacer Arm 20.4 Å** | • Use the EDC crosslinker to couple this analog to carboxyl (–COOH) groups<br>• Water-soluble |
| **Amine-PEG$_3$-Biotin** |  <br>**Amine-PEG$_3$-Biotin**<br>**MW 418.55**<br>**Spacer Arm 22.9 Å** | • Use the EDC crosslinker to couple this analog to carboxyl (–COOH) groups<br>• Water-soluble<br>• Longer chain length for reduction of steric hindrance |

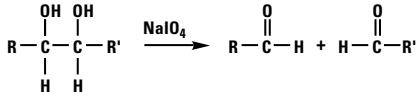
Amine-$PEG_2$-Biotin, Amine-$PEG_3$-Biotin and Pentylamine-Biotin are amine-derivatized biotin molecules that can be reacted with carboxyl groups. In addition, any of the hydrazide-derivatized biotin molecules can be reacted with carboxyls under identical conditions. The reaction is most often mediated by EDC, a water-soluble carbodiimide that activates carboxyl groups to bind to the $–NH_2$ group on the biotinylation reagent. Using this strategy may result in some polymerization of the peptide or protein if the molecule has both carboxyls and primary amines on its surface. The extent of polymerization can be minimized by decreasing the amount of EDC and/or increasing the amount of the biotin reagent used in the reaction.

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Cernuda-Morollon, E., *et al.* (2001). *J. Biol. Chem.* **276**, 35530-35536.<br>• Liu, Y., *et al.* (1999). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14694-14699. | **21345** | **EZ-Link Pentylamine-Biotin** | 50 mg |
| • Fezza, F., *et al.* (2008) *J. Lipid Res.* **49**, 1216-1223.<br>• Cui, B., *et al.* (2007) *Proc. Nat. Acad. Sci. USA.* **104**, 13666-13671.<br>• Pihlajamaa, T., *et al.* (2004) *J. Biol. Chem.* **279**, 24265-24273. | **21346** | **EZ-Link Amine-$PEG_2$-Biotin** | 50 mg |
| • Maguire, B. A., *et al.* (2008) *RNA.* **14**, 188-195. | **21347** | **EZ-Link Amine-$PEG_3$-Biotin** | 50 mg |

# Carbohydrate/Aldehyde-Reactive Biotinylation Reagents

### Carbohydrate/Aldehyde-Reactive Biotinylation Reagents

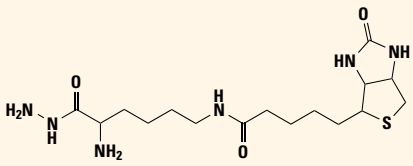
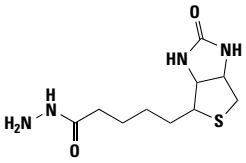
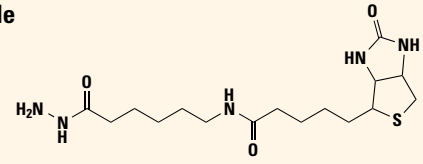
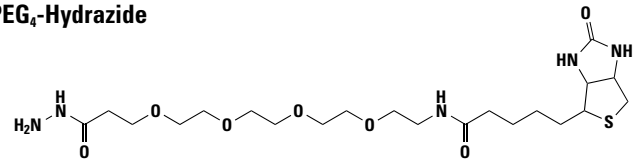
Another common target for protein modification is the carbohydrate portion of glycoproteins, which can be reacted with hydrazide-derivatives of biotin. Oxidative treatment of glycoproteins using 10 mM periodate is used to generate reactive aldehydes from the cis-diols of a variety of carbohydrate moieties.




**Oxidation of a *cis*-diol to an aldehyde**

An aldehyde can be reacted specifically with a hydrazide group at pH 4-6, forming a stable hydrazone linkage. Sialic acid residues on glycoproteins can be specifically oxidized with sodium periodate ($NaIO_4$) under mild conditions. At 1 mM periodate and a temperature of 0°C, oxidation is restricted primarily to sialic acid residues. Sialic acid residues also can be biotinylated with hydrazide derivatives by pretreatment with neuraminidase to generate galactose groups. The galactose and *N*-acetylgalactosamine residues on whole cells can be selectively biotinylated with Biotin-Hydrazides by further treatment with galactose oxidase. This enzyme will convert the primary hydroxyl groups on these sugars to their corresponding aldehydes.

## Thermo Scientific EZ-Link Carbohydrate/Aldehyde-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Biocytin-Hydrazide** |  <br>**Biocytin-Hydrazide**<br>**MW 386.51**<br>**Spacer Arm 19.7 Å** | • Can be used to label DNA and RNA through cytosine residues<br>• More water-soluble than Biotin-LC-Hydrazide |
| **Biotin-Hydrazide** |  <br>**Biotin-Hydrazide**<br>**MW 258.34**<br>**Spacer Arm 15.7 Å** | • Carbohydrate reactive<br>• Must be dissolved in DMSO before adding to aqueous buffer |
| **Biotin-LC-Hydrazide** |  <br>**Biotin-LC-Hydrazide**<br>**MW 371.50**<br>**Spacer Arm 24.7 Å** | • Use the EDC crosslinker to couple this analog to carboxyl (–COOH) groups<br>• Water-soluble<br>• Longer chain length for reduction of steric hindrance |
| **Biotin-PEG$_4$-Hydrazide** |  <br>**Biotin-PEG$_4$-Hydrazide**<br>**MW 505.63**<br>**Spacer Arm 31.3 Å** | • Water-soluble analog of Biotin-LC-Hydrazide<br>• PEG-based spacer arm increases solubility of labeled molecules |

Mild oxidation of an immunoglobulin with sodium periodate produces reactive aldehydes from the carbohydrate moieties on the Fc portion of the antibody, which then can be alkylated by a hydrazide. This approach is advantageous for use with antibodies because they become biotinylated in a manner that maintains immunological reactivity. This is an ideal method for biotinylating polyclonal antibodies because they are heavily glycosylated. Monoclonal antibodies may be deficient in glycosylation and success with this method will depend on the extent of glycosylation for a particular antibody.

Temperature, pH of oxidation and the periodate concentration all affect the reaction with hydrazide derivatives of biotin. Also, because glycosylation varies with each protein, optimum conditions must be determined for each glycoprotein. Each glycoprotein preparation has an optimum pH for oxidation and for the hydrazide-mediated biotinylation. Tris, or other primary amine-containing buffers, are not recommended for use in either the oxidation or biotinylation steps because these buffers react with aldehydes, quenching their reaction with hydrazides.

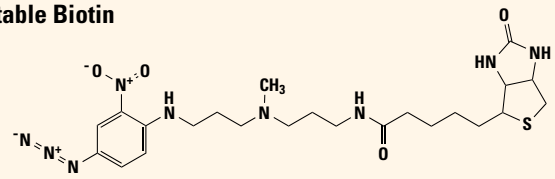
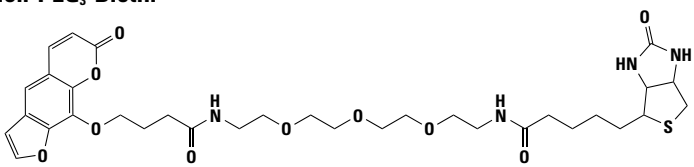
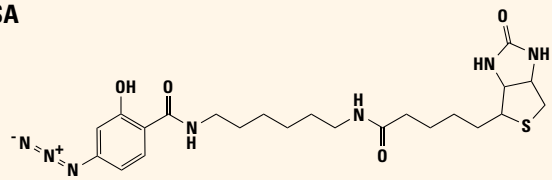
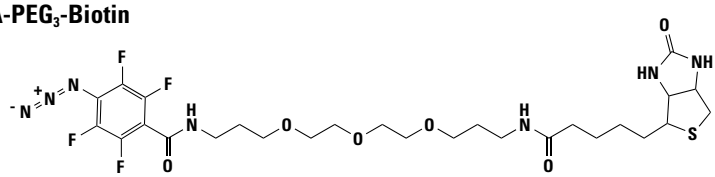
| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Bayer, E.A. *et al.* (1988). *Anal. Biochem.* **170**, 271-281.<br>• Reisfeld, A., *et al.* (1987). *Biochem. Biophys. Res. Commun.* **142**, 519-526.<br>• Roffman, E., *et al.* (1986). *Biochem. Biophys. Res. Commun.* **136**, 80-85. | **28020** | **EZ-Link Biocytin-Hydrazide** | 25 mg |
| • Edwards, S.W., *et al.* (1999). *J. Biol. Chem.* **274**, 16331-16336.<br>• Reisfeld, A., *et al.* (1987). *Biochem. Biophys. Res. Commun.* **142**, 519-526. | **21339** | **EZ-Link Biotin-Hydrazide** | 100 mg |
| • Araga, S., *et al.* (1999). *J. Immunol.* **163,** 476-482<br>• Kahne, T. and Ansorge, S. (1994). *J. Immunol. Methods* **168,** 209-218.<br>• Luk, J.M., *et al.* (1995). *Anal. Biochem.* **232,** 217-224<br>• Scott, M.G., *et al.* (2000). *J. Immunol.* **164,** 549-553.<br>• Yu, Q. and Toole, B.P. (1995). *Biotechniques* **19,** 122-129. | **21340** | **EZ-Link Biotin-LC-Hydrazide** | 50 mg |
| | **21360** | **EZ-Link Biotin-PEG$_4$-Hydrazide** | 50 mg |

# Photo-Reactive Biotinylation Reagents

### Photo-Reactive Biotinylation Reagents

Proteins, peptides and other molecules that do not contain any of the reactive functional groups mentioned previously may be labeled using a photo-reactive biotinylation reagent. These reagents contain an aryl azide group that is chemically inert until it is exposed to ultraviolet light, causing the formation of a short-lived, reactive aryl nitrene. The half-life of this aryl nitrene intermediate is on the order of $10^{-4}$ seconds. The aryl nitrene reacts rapidly and nonselectively with electron dense sites by addition into double bonds or insertion into active hydrogen bonds. If the aryl-nitrene fails to react, it undergoes ring expansion and becomes reactive toward nucleophiles such as primary amines and sulfhydryls. Photoactivation and insertion into another molecule can be performed in a wide variety of buffer conditions. However, acidic conditions and reducing agents should be avoided because they may inactive the aryl azide group.

## Thermo Scientific EZ-Link Photo-Reactive Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Photoactivatable Biotin** |  **Photoactivatable Biotin** MW 533.65 Spacer Arm 30.0 Å | • Covalent attachment occurs in the presence of UV light (350-370 nm)<br>• Attachment is nonspecific<br>• Must be dissolved in DMSO or DMF before use<br>• Reaction buffer should be above pH 6 |
| **Psoralen-PEG$_3$-Biotin** |  **Psoralen-PEG$_3$-Biotin** MW 688.79 Spacer Arm 36.9 Å | • Water-soluble<br>• Much higher coupling yield than achieved with photoactivatable biotin<br>• Psoralen moiety reacts with the 5,6 double bond in thymine- and other pyrimidine-containing bases<br>• Covalent attachment occurs in the presence of UV light (>350 nm) for 10-30 minutes<br>• Great for biotinylating DNA/RNA probes<br>• Ultrahigh sensitivity relative to radiolabeled probes (<100 femtograms)<br>• Doesn't interfere with hybridization |
| **Biotin-LC-ASA** |  **Biotin-LC-ASA** MW 503.62 Spacer Arm 29.9 Å | • Iodinatable and photoactivatable<br>• Useful for biotinylation of nucleic acids |
| **TFPA-PEG$_3$-Biotin** |  **TFPA-PEG$_3$-Biotin** MW 663.69 Spacer Arm 33.4 Å | • Label almost any molecule with biotin<br>• TFPA is more efficient than other photo-reactive groups<br>• Long, PEG-based spacer arm reduces steric hindrance and increases conjugate solubility |

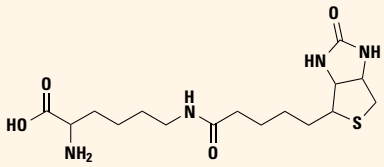
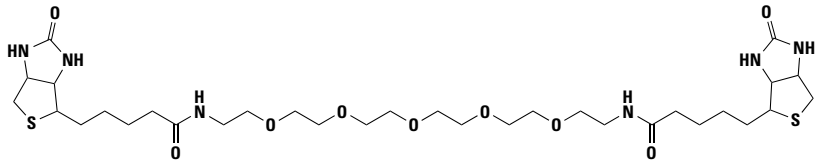
Photoactivatable Biotin, Biotin-LC-ASA , Psoralen-$PEG_3$-Biotin and TFPA-$PEG_3$-Biotin each contain a photoactivatable group. When exposed to UV light, they become activated and insert nonspecifically into nearby molecules. These reagents may be used to label proteins and peptides, but they are also useful in labeling DNA, RNA and other molecules that do not contain any readily reactive functional groups.

Psoralen-$PEG_3$-Biotin contains a reactive psoralen group and is designed to efficiently label nucleic acids. The psoralen group intercalates into the helix of DNA, allowing it to label efficiently and selectively. The psoralen can also stack along with the bases of single-stranded DNA or RNA increasing labeling efficiency and selectivity. Upon photoactivation, psoralen forms a crosslink with the 5,6 double bond of pyrimidine bases and its presence does not interfere with hybridization.

| References | Ordering Information | | |
|---|---|---|---|
| | Product # | Description | Pkg. Size |
| • Lacey, E. and Grant, W.N., (1987). *Anal. Biochem.* **163**, 151-158.<br>• Smith, J.S., *et al.* (1999). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8855-8860. | **29987** | **EZ-Link Photoactivatable Biotin** | 0.5 mg |
| • Cimono, G.D., *et al.* (1985). *Annu. Rev. Biochem.* **54**, 1151-1193.<br>• Wassarman, D.A. (1993). *Mol. Biol. Rep.* **17**, 143-151. | **29986** | **EZ-Link Psoralen-$PEG_3$-Biotin** | 5 mg |
| • Kotani, N., *et al.* (2008) *Proc. Nat. Acad. Sci. USA.* **105**, 7405-7409.<br>• Koraha, J., *et al.* (2005) *Clin. Diagn. Lab. Immunol.* **12**, 1292-1297. | **29982** | **EZ-Link Biotin-LC-ASA** | 2 mg |
| | **21303** | **EZ-Link TFPA-$PEG_3$-Biotin** | 25 mg |

# Specialty Biotinylation Reagents

## EZ-Link Specialty Biotinylation Reagents

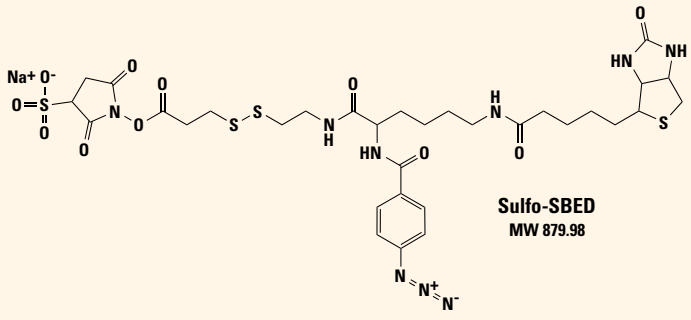
| Reagent | Structure | Highlights |
|---|---|---|
| **Biocytin** |  **Biocytin** MW 372.48 Spacer Arm 20.1 Å | • Useful for synthesizing long-chain biotinylation reagents |
| **$PEG_5$-Biotin Dimer** |  **PEG5-Biotin Dimer** MW 732.95 Spacer Arm 43.4 Å | • Long spacer arm biotin dimer for crosslinking avidin molecules<br>• Can be used to increase signal in assays using the avidin-biotin system<br>• Water-soluble |

### Label Transfer with Specialty Biotinylation Reagents

The label transfer technique is gaining popularity as a valuable strategy for identifying relevant protein interactions. A label transfer reagent consists of a reactive group that can be bound to a protein of interest, a second reactive group that can be bound to another protein that interacts with the protein of interest, a cleavable spacer arm, and a label that can be used to purify or identify the labeled protein. One advantage of the label transfer technique over other *in vitro* methods for studying protein interactions is its ability to identify molecules with only a weak or transient interaction. These transiently interacting molecules are generally not co-purified using immunoprecipitation or pull-down methods because of the number of washing steps and the time involved.

Sulfo-SBED contains an amine-reactive NHS-ester, a photo-reactive group, a cleavable disulfide linkage, and a biotin label. Available amine groups on a purified protein are first reacted with the NHS-ester of Sulfo-SBED. The labeled protein is then

## Thermo Scientific Pierce Specialty Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Sulfo-SBED Biotin Label Transfer Reagent** |  **Sulfo-SBED** MW 879.98 | • Photo-reactive aryl nitrene group couples to protein through C-H bonds when activated at 300-350 nm<br>• Sulfo-NHS ester reacts with amines to form stable amide bonds at pH 7-9<br>• Spacer arm contains a biotin group to allow recovery of conjugate on immobilized avidin column<br>• Cleavable disulfide bond in the spacer arm allows molecules to be detached from each other |

## References


- Baqui, M., *et al.* (2003). *J. Biol. Chem.* **278**, 1206-1211.
- Cimono, G.D., *et al.* (1985). *Annu. Rev. Biochem.* **54**, 1151-1193.
- Wassarman, D.A. (1993). *Mol. Biol. Rep.* **17**, 143-151.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **28022** | **EZ-Link Biocytin** | 100 mg |
| **22020** | **EZ-Link $PEG_5$-Biotin Dimer** | 50 mg |

incubated with either a pure preparation of a binding partner or with a complex mixture such as a lysate to search for a binding partner. Complexes form through protein:protein interactions and the sample is exposed to UV light in the 300-366 nm range. The photo-reactive moiety inserts itself into a nearby bond trapping the interacting protein in a covalent complex. By reducing the disulfide linkage, the label can be transferred to the interacting protein. Then the protein can be purified or detected using the biotin label.

### Sulfo-SBED Applications include:

- Searching for putative binding partners
- Interaction mapping
- Study of complex assembly mechanisms
- Defining docking site and co-factor requirements of interactions
- Studying refolding interactions
- Detecting low abundance receptors
- Evaluating drug-receptor interactions

## References


- Alley, S.C., *et al.* (2000). *J. Am. Chem. Soc.* **122**, 6126-6127.
- Daum, J.R., *et al.* (2000). *Curr. Biol.* **10**, 850-852.
- Geselowitz, D.A. and Neumann, R.D., (1995). *Bioconjugate Chem.* **6**, 502-506.
- Horney, Mark J., *et al.* (2001). *J. Biol. Chem.* **276**, 2880-2889.
- Ilver, D., *et al.* (1998). *Science* **279**, 373-377.
- Ishmael, F.T., *et al.* (2002). *J. Biol. Chem.* **277**, 20555-20562.
- Jacobson, *et al.* (1995). *Life Sci.* **56**, 823-830.
- Kleene, R., *et al.* (2000). *Biochemistry* **39**, 9893-9900.
- Minami, Y., *et al.* (2000). *J. Biol. Chem.* **275**, 9055-9061.
- Muroi, M., *et al.* (2002). *J. Biol. Chem.* **277**, 42372-42379.
- Neely, K.E., *et al.* (2002). *Mol. Cell. Biol.* **22**, 1615-1625.
- Sharma, K.K., *et al.* (2000). *J. Biol. Chem.* **275**, 3767-3771.
- Trotman, L.C., *et al.* (2001). *Nat. Cell Biol.* **3**, 1092-1100.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **33033** | **Sulfo-SBED Biotin Label Transfer Reagent†** | 10 mg |
| **33034** | **Sulfo-SBED Biotin Label Transfer Reagent** | 8 x 1 mg |
| **33073** | **Sulfo-SBED Biotin Label Transfer Kit-Western Blot Application**<br>*Sufficient reagents for 8 label transfer reactions*<br>Includes: Sulfo SBED<br>Phosphate Buffered Saline<br>Label Transfer Buffer (20X)<br>Streptavidin-Horseradish Peroxidase Conjugate<br>Dithiothreitol (DTT)<br>Slide-A-Lyzer® MINI Dialysis Units Plus Float, 10K MWCO | Kit<br><br><br><br><br>8 x 1 mg<br>1 pack<br>200 ml<br>0.1 mg<br><br>8 x 7.7 mg<br>10 units/pkg. |

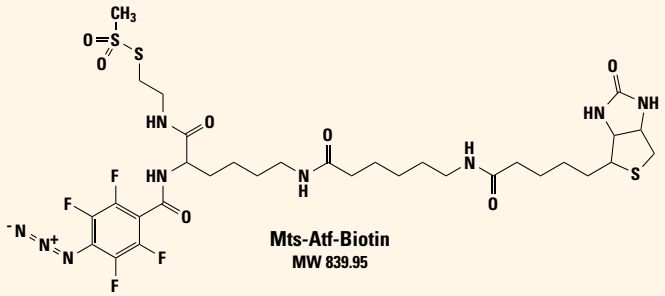
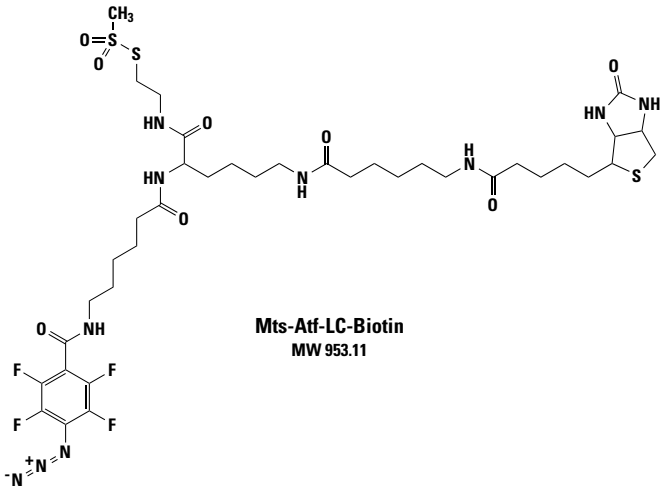
*† See patent information.*

# Specialty Biotinylation Reagents

### Sulfhydryl-directed, photoactivated biotin label transfer reagents

Thermo Scientific Mts-Atf-Biotin Reagents are useful for determining specific components of a protein interaction. A known purified protein can be labeled specifically at native or engineered sulfhydryl sites, and then allowed to bind with its interactors. Exposure to UV-light activates the tetrafluorophenyl azide group, resulting in conjugation of the interacting proteins. After affinity purification, the

## Thermo Scientific Pierce Specialty Biotinylation Reagents

| Reagent | Structure | Highlights |
|---|---|---|
| **Mts-ATF-Biotin Label Transfer Reagent** |  **Mts-Atf-Biotin** MW 839.95 | • Sulfo-NHS ester reacts with primary amines at pH 7-9<br>• Aryl azide conjugates randomly when activated with UV-light<br>• Cleavable disulfide bond allows biotin label transfer<br>• Discover protein interactions without radiolabeling |
| **Mts-ATF-Biotin-LC Label Transfer Reagent** |  **Mts-Atf-LC-Biotin** MW 953.11 | • Sulfo-NHS ester reacts with primary amines at pH 7-9<br>• Aryl azide conjugates randomly when activated with UV-light<br>• Cleavable disulfide bond allows biotin label transfer<br>• Discover protein interactions without radiolabeling |

captured interactors can be released from the original known protein "bait" by reduction of the disulfide bond, leaving a biotin label on the interactor.

## References


• Layer, G. *et al.* (2007). *J. Biol. Chem.* **282**, 13342-13350.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **33083** | **Mts-ATF-Biotin Label Transfer Reagent** | 5 mg |
| **33093** | **Mts-ATF-Biotin-LC Label Transfer Reagent** | 5 mg |

## Pierce Biotin Quantitation Kit

*A convenient, accurate method for determining the degree of biotinylation.*

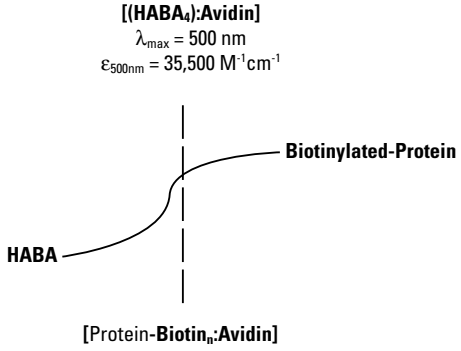
Determine the molar ratio of biotin incorporated into an antibody using the HABA-Avidin method. The HABA dye (2-hydroxyazo-benzene-4'-carboxylic acid) binds to avidin to produce a yellow-orange colored complex that absorbs at 500 nm. Free biotin present in solution with this avidin-HABA complex will displace the HABA dye and cause the absorbance to decrease in proportion to the amount of biotin.

The amount of biotin present can be calculated directly from the decreased absorbance at 500 nm. The Thermo Scientific Pierce Biotin Quantitation Kit contains pre-measured doses of the Avidin-HABA mixture and Biotinylated HRP positive control to simplify reagent preparation and minimize the amount of waste generated. The convenient assay can be performed either in a cuvette or in a microplate, and the math is simplified using a calculator on the our web site. Thermo Scientific Pierce Avidin and Biotinylated HRP are also available separately in larger quantities.

### How does this biotinylation assay work?

The $HABA_4$:Avidin complex is at the core of this displacement assay that can estimate the extent of protein biotinylation. HABA dye binds to avidin to form a complex that absorbs strongly at 500 nm with an extinction at that wavelenth of 35,000 $M^{-1}cm^{-1}$.

The assay is based on the decrease in absorbance of the [($HABA_4$):Avidin] complex when HABA is displaced from the complex by biotin.




### Highlights:

- HABA-avidin complex can be used over a wide range of pH and salt concentrations
- Amount of biotin can be calculated directly from the decreased absorbance at 500 nm complexing with the HABA dye

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **28005** | **Biotin Quantitation Kit**<br>Includes: No-Weigh HABA-Avidin Premix<br>Biotinylated HRP | 24 assays<br>24 tubes<br>5 mg |
| **28010** | **HABA**<br>(2-[4'-Hydroxyazobenzene]-benzoic acid) | 10 g |
| **21121** | **Avidin 10** | 10 mg |
| **29129** | **D-Biotin** | 1 g |
| **29139** | **Biotinylated-HRP** | 5 mg |

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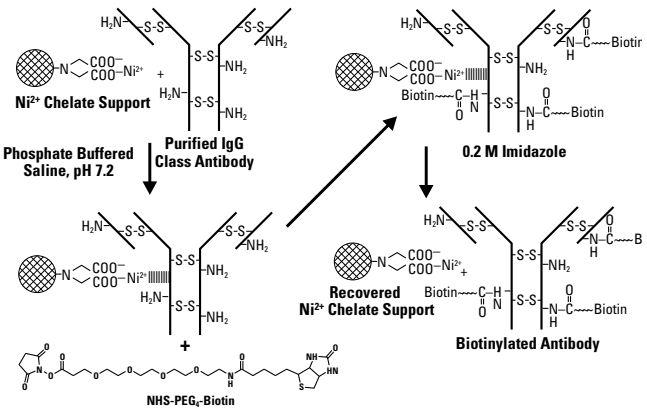
## EZ-Link Solid-Phase Biotinylation Kits

*An easier way to biotinylate IgG antibodies.*

This innovative antibody-labeling system uses nickel-chelated agarose to temporarily immobilize antibody molecules via their histidine-rich Fc regions. Once held in place on the gel, the antibody can be biotinylated at either sulfhydryl groups (after mild reduction or disulfide bonds) or primary amines. Excess labeling reagent and byproducts are then washed away before recovering the labeled and purified antibody from the gel using a mild imidazole solution. No gel filtration or dialysis is needed. Four kits are available for small (0.1-1 mg) or large (1-10 mg) antibody samples using either amine-directed (NHS ester) or sulfhydryl-directed (maleimide) labeling reagents.

### Highlights:

- **Fast labeling and purification** – the entire procedure takes only one hour (two hours for sulfhydryl labeling kits)
- **Easy removal of spent and excess labeling reagent** – simply wash away the reaction byproducts – no need for dialysis or gel filtration
- **No dilution effects** – solid-phase method allows initially dilute antibodies to be recovered in a smaller volume after labeling
- **Optimized protocols** – specific protocols for antibody ensure appropriate level of labeling (2-5 biotins per antibody molecule), minimizing possibility of inactivation caused by overlabeling
- **Sufficient reagents for eight biotin-labeling experiments** – Thermo Scientific No-Weigh Single-Dose Microtube Packaging ensures that the biotin reagent is fully active for eight separate experiments




**Summary of Solid-Phase Biotinylation Protocol**.

**Step 1.** Immobilize the IgG
a. 1 ml Ni-IDA column (for 1-10 mg of IgG)
b. Nickel Chelated Disc (for 0.1-1 mg IgG)

**Step 2.** Add the labeling reagent(s) to the immobilized IgG
a. NHS-$PEG_4$ Biotin for amine-directed reactions
b. TCEP, followed by Maleimide-$PEG_2$-Biotin for sulfhydryl-directed reactions

**Step 3.** Elute the biotinylated IgG with 0.2 M imidazole

### Two kit sizes and labeling chemistries:

| Antibody Sample Size | Amine-directed Labeling (NHS-$PEG_4$-Biotin) | Sulfhydryl-directed Labeling (Maleimide-$PEG_2$-Biotin) |
|---|---|---|
| 0.1-1 mg IgG | Product # 21450 | Product # 21930 |
| 1-10 mg IgG | Product # 21440 | Product # 21920 |

These kits contain our exclusive No-Weigh Single-Dose Microtube Packaging. A single sealed microtube containing 2 mg of reagent is reconstituted for each biotinylation. The exclusive packaging allows access to fresh reagent on-demand for each solid-phase biotinylation reaction.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **21440** | **NHS-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column**<br>*Biotinylates antibodies and other proteins that bind to the nickel-chelated support provided. A 1 ml column biotinylates 1-10 mg of antibody and can be re-used 10 times.*<br>Includes: Immobilized Nickel Chelated Column<br>BupH™ Phosphate Buffered Saline<br>NHS-$PEG_4$-Biotin<br>4 M Imidazole Stock Solution | Kit<br><br><br>1 ml<br>1 pack<br>8 x 2 mg<br>5 ml |
| **21450** | **NHS-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns**<br>*Biotinylate antibodies and other proteins that bind to the rehydrated nickel-chelated discs provided. Each disc can biotinylate 100-1,000 µg of antibody.*<br>Includes: Nickel Chelated SwellGel® Discs†<br>Mini-Spin Columns<br>Microcentrifuge Tubes (2 ml)<br>BupH Phosphate Buffered Saline<br>NHS-$PEG_4$-Biotin<br>4 M Imidazole Stock Solution | Kit<br><br><br><br>10 pack<br>10 pack<br>30 pack<br>1 pack<br>8 x 2 mg<br>5 ml |
| **21920** | **Maleimide-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column**<br>*Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. A 1 ml column biotinylates 1-10 mg of antibody and can be re-used 10 times.*<br>Includes: Bond-Breaker® TCEP Solution, Neutral pH<br>Immobilized Nickel Chelated Column<br>BupH Tris Buffered Saline<br>Maleimide-$PEG_2$-Biotin<br>4 M Imidazole Stock Solution | Kit<br><br><br><br>5 ml<br>1 ml<br>1 pack<br>8 x 2 mg<br>5 ml |
| **21930** | **Maleimide-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns**<br>*Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. Each disc can biotinylate 100-1,000 µg of antibody.*<br>Includes: Nickel Chelated SwellGel Discs<br>Bond-Breaker TCEP Solution, neutral pH<br>Mini-Spin Columns<br>Microcentrifuge Tubes (2 ml)<br>BupH Tris Buffered Saline<br>Maleimide-$PEG_2$-Biotin<br>4 M Imidazole Stock Solution | Kit<br><br><br><br>10 pack<br>5 ml<br>10 pack<br>30 pack<br>1 pack<br>8 x 2 mg<br>5 ml |

*† See patent information.*

**References**
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## Cell Surface Protein Isolation Kit

*Convenient biotinylation and isolation of cell surface proteins for Western blot analysis.*

The Thermo Scientific Cell Surface Protein Isolation Kit is a complete kit for the convenient biotinylation and isolation of mammalian cell surface proteins, specifically targeting cell surface proteins to the exclusion of intracellular proteins. The kit efficiently labels proteins with accessible lysine residues and sufficient extracellular exposure.

The isolation procedure uses a cell-impermeable, cleavable biotinyla-tion reagent (Sulfo-NHS-SS-Biotin) to label surface proteins at exposed primary amines. Cells are then harvested and lysed, and the labeled surface proteins are affinity-purified using NeutrAvidin Agarose Resin. The isolated cell surface proteins contain a small, nonreactive tag of the originally labeled primary amines but are no longer biotinylated (biotin remains bound to the resin).

### Highlights:

- **Isolates cell surface proteins** – reduces complexity of total cellular protein
- **Efficiently recovers labeled proteins** – cleavable biotin allows for nearly 100% recovery of isolated cell surface proteins
- **Convenience** – all reagents are provided in one kit, along with complete instructions for labeling, cell lysis and purification of cell surface membrane proteins
- **Western blotting applications** – proteins recovered in SDS-PAGE buffer are loaded directly onto polyacrylamide gels
- **Robust system** – protocol designed for diverse cell lines, including NIH 3T3, HeLa, C6 and A431

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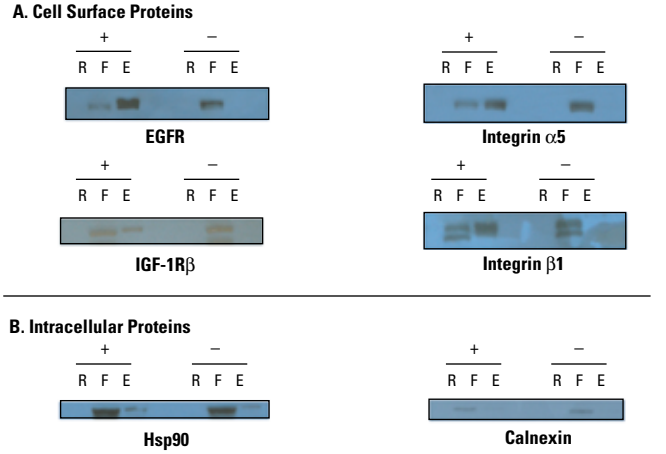
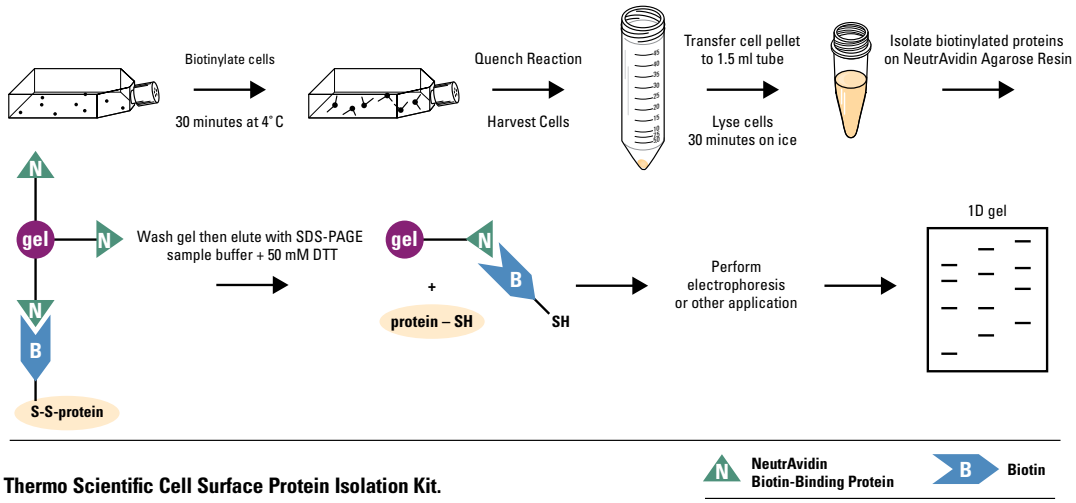



**Protein isolation is specific to cell surface proteins.** Panels are Western blot results for known cell surface proteins (Panel A) and intracellular proteins (Panel B) from HeLa cells tested with the Cell Surface Protein Isolation Kit. Plus symbol (+) denotes results for cells treated with the Sulfo-NHS-SS-Biotin reagent; minus symbol (-) denotes results for cells that were not treated with the biotin reagent but were otherwise carried through the kit procedure. Lanes are no-sample resin-control (R), flow-through (F) and eluted (E) fractions. Presence of target cell surface proteins in the plus-E and minus-F conditions indicate successful isolation with the kit. Presence of intracellular proteins in F condition of both plus and minus conditions indicates that the labeling and purification procedure is specific to cell surface proteins.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **89881** | **Cell Surface Protein Isolation Kit**<br>*Sufficient reagents and accessories for eight experiments, each involving four T75 flasks of confluent cells.* | Kit |
| | Includes: EZ-Link Sulfo-NHS-SS-Biotin | 8 x 12 mg vials |
| | Quenching Solution | 16 ml |
| | Lysis Buffer | 4.5 ml |
| | NeutrAvidin Agarose | 2.25 ml |
| | Wash Buffer | 34 ml |
| | Dithiothreitol (DTT) | 8 x 7.7 mg |
| | BupH Phosphate Buffered Saline | 2 packs |
| | BupH Tris Buffered Saline | 1 pack |
| | Spin Columns and Accessories | |




**Procedure for the Thermo Scientific Cell Surface Protein Isolation Kit.**

## Far-Western Blotting

The technique of Western blotting has been adapted for use in the search for protein:protein interactions. A far-Western blot uses a tagged protein other than an antibody to probe a membrane. The probe recognizes and binds to proteins on the membrane through protein:protein interactions. Thus a signal generated from a band on the membrane indicates the presence of a protein that interacts with the probe at that apparent molecular weight. This method can be used to search for unknown protein interactions or to confirm putative interactions. The Thermo Scientific Pierce Far-Western Biotinylated Protein:Protein Interaction Kit uses a biotin-tagged protein as the probe for far-Western blotting.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **23500** | **Far-Western Biotinylated-Protein Interaction Kit**<br>*Materials and methods for the discovery, in-gel or on-membrane, of protein interactions using a biotinylated bait protein as the probe.* | 10 mini-gels |
| | Includes: Streptavidin-HRP | 0.1 mg |
| | Dilution Buffer (10X) | 50 ml |
| | Phosphate Buffered Saline | 17 packs |
| | 10% Tween®-20 | 6 x 10 ml ampules |
| | Pierce In-Gel Stable Peroxide | 55 ml |
| | Pierce In-Gel Luminol Enhancer | 55 ml |
| | Cellophane Exposure Sheets | 10 pack |

## Pull-Down Kit for Biotinylated Proteins

A pull-down assay is an *in vitro* method for identifying or confirming protein:protein interactions using a purified and immobilized bait protein. The immobilized bait protein is used as a specific affinity ligand (much as an antibody is used in immunoprecipitation) to capture interacting "prey" proteins from a lysate or other complex mixture. The Thermo Scientific Pierce Pull-Down Biotinylated-Protein:Protein Interaction Kit requires a biotin-tagged protein for the bait and all other reagents are supplied with the kit.

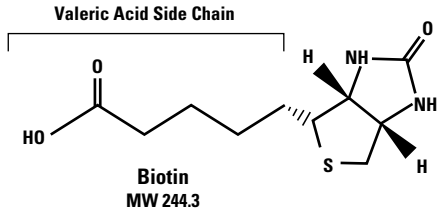
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **21115** | **Pull-Down Biotinylated-Protein Interaction Kit**<br>*Sufficient materials for conducting 25 pull-down assays using a purified and biotinylated protein as the bait.* | Kit |
| | Includes: Immobilized Streptavidin | 1.5 ml settled gel |
| | BupH Tris Buffered Saline | 1 pack (makes 500 ml) |
| | Biotin Blocking Buffer | 15 ml |
| | Wash Buffer (Acetate, pH 5.0) | 100 ml |
| | Elution Buffer (pH 2.8) | 50 ml |
| | Spin Cup Columns Accessory Pack | 27 columns |
| | Collection Tubes and Caps Accessory Pack | 200 x 2 ml tubes, graduated |

# Avidin-Biotin Binding

## Biotin

Biotin, also known as vitamin H, is a small molecule (MW 244.3) that is present in tiny amounts in all living cells. The valeric acid side chain of the biotin molecule can be derivatized to incorporate various reactive groups that are used to attach biotin to other molecules. Once biotin is attached to a molecule, the molecule can be affinity-purified using an immobilized version of any biotin-binding protein. Alternatively, a biotinylated molecule can be immobilized through interaction with a biotin-binding protein, then used to affinity-purify other molecules that specifically interact with it. We offer biotin-labeled antibodies and a number of other biotinylated molecules, as well as a broad selection of biotinylation reagents to label any protein.




## Biotin-Binding Proteins

**Avidin** – The extraordinary affinity of avidin for biotin allows biotin-containing molecules in a complex mixture to be discretely bound with avidin. Avidin is a glycoprotein found in the egg white and tissues of birds, reptiles and amphibians. It contains four identical subunits having a combined mass of 67,000–68,000 daltons. Each subunit consists of 128 amino acids and binds one molecule of biotin. The extent of glycosylation on avidin is high; carbohydrate accounts for about 10% of the total mass of the tetramer. Avidin has a basic isoelectric point (pI = 10–10.5) and is stable over a wide range of pH and temperature. Extensive chemical modification has little effect on the activity of avidin, making it especially useful for protein purification. However, because of its carbohydrate content and basic pI, avidin has relatively high nonspecific binding properties.

**Streptavidin** – Another biotin-binding protein is streptavidin, which is isolated from *Streptomyces avidinii* and has a mass of 75,000 daltons. In contrast to avidin, streptavidin has no carbohydrate and has a mildly acidic pI (5.5). Thermo Scientific Pierce Streptavidin is a recombinant form having a mass of 53,000 daltons and a near-neutral pI. Streptavidin is much less soluble in water than avidin. There are considerable differences in the composition of avidin and streptavidin, but they are remarkably similar in other respects. Streptavidin is also a tetrameric protein, with each subunit binding one molecule of biotin with affinity similar to that of avidin. Guanidinium chloride will dissociate avidin and streptavidin into subunits, but streptavidin is more resistant to dissociation. Streptavidin contains an RYD sequence similar to the RGD sequence that binds cell surface receptors. The RYD sequence can cause background in some applications.

**NeutrAvidin Protein** – We also offer a deglycosylated version of avidin, known as NeutrAvidin Protein, with a mass of approximately 60,000 daltons. As a result of carbohydrate removal, lectin binding is reduced to undetectable levels, yet biotin-binding affinity is retained because the carbohydrate is not necessary for this activity. NeutrAvidin Protein offers the advantages of a near-neutral pI (6.3) to minimize nonspecific adsorption, along with lysine residues that remain available for derivatization or conjugation. NeutrAvidin Protein yields the lowest nonspecific binding among the known biotin-binding proteins due to its near-neutral pI and lack of both carbohydrate and RYD sequence.

**Strength of Avidin-Biotin Interaction** – The avidin-biotin complex is the strongest known noncovalent interaction ($K_a = 10^{15}\ M^{-1}$) between a protein and ligand. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by extremes of pH, temperature, organic solvents and most denaturing agents. These features of avidin – features that are shared by streptavidin and NeutrAvidin Protein – make immobilized forms of the biotin-binding proteins particularly useful for purifying biotin-labeled proteins or other molecules. However, the strength of the interaction and its resistance to dissociation make it difficult to elute bound proteins from an immobilized support. Harsh, denaturing conditions (8 M guanidine•HCl, pH 1.5 or boiling in SDS-sample loading buffer) are required to efficiently dissociate avidin-biotin complexes. Such conditions damage the support irreversibly so that it cannot be reused, and denature the eluted proteins so that they do not maintain any biological activity.

Because of these binding and elution properties, purifications based on avidin-biotin affinity are reserved primarily for small-scale procedures involving immediate analysis of the eluted sample by reducing SDS-PAGE or other denaturing method. On the other hand, it is possible to take advantage of the strong avidin-biotin binding properties in immunoprecipitation (IP) and pull-down procedures because the immunoprecipitated "prey" protein can be recovered using elution conditions that will not also elute the biotinylated antibody or "bait" protein. In some situations, it may be most appropriate to use a cleavable biotinylation reagent to label the target molecule so that it may be recovered from its bound state to immobilized avidin by specific cleavage of the spacer arm between biotin and target molecule rather than by elution of biotin from avidin.

**Monomeric Avidin** – Immobilized Monomeric Avidin was developed to allow the purification of fully functional biotinylated proteins. Unlike other biotin-binding proteins that require harsh, denaturing conditions to elute and recover bound molecules, Monomeric Avidin binds reversibly to biotin and allows gentle elution and recovery of biotinylated molecules using a solution of 2 mM biotin to compete for the biotin-binding sites. This makes it possible to harness the avidin-biotin interaction as a purification tool to recover functional proteins and other biological molecules.

## Biotin-Binding Products

Each of the four biotin-binding proteins discussed is available in a variety of immobilized formats. The support resin used for our Immobilized Avidin, Streptavidin and NeutrAvidin Protein is a crosslinked 6%, beaded agarose. Our Immobilized Monomeric Avidin uses a crosslinked 4% beaded agarose. Thermo Scientific UltraLink Biosupport is a durable, polyacrylamide-based resin with a high surface area, large pore volume and low nonspecific binding. It is suitable for pressures up to 100 psi and linear flow rates up to 3,000 cm/hour. A biotin-binding protein immobilized on beaded agarose or our UltraLink® Biosupport can be used for affinity purification in a column or batch method. NeutrAvidin Protein and Streptavidin are also available bound to polystyrene microplates along with a dried blocking buffer. These 96-well plates are offered in transparent, white or black plates to accommodate a variety of assay types. The plates come in two forms – regular and high-binding capacity. The high-binding capacity plates contain more of the immobilized NeutrAvidin Protein or Streptavidin and are ideal for binding large amounts of small, biotin-containing molecule (e.g., a biotinylated peptide). Streptavidin immobilized on MagnaBind Magnetic Beads is an excellent tool for cell-sorting applications.

## A Comparison of Biotin-Binding Proteins

The strong association between avidin and biotin can be used in the field of affinity separations. By attaching avidin to a solid support, a biotinylated product can be anchored to the same solid support. The attachment is stable over a wide range of pH, salt concentrations and temperatures. To dissociate biotin from avidin, 8 M guanidine•HCl, pH 1.5 or boiling in SDS-PAGE sample buffer must be used.

| | Avidin | Streptavidin | NeutrAvidin Protein |
|---|---|---|---|
| **Molecular Weight** | 67 kDa | 53 kDa | 60 kDa |
| **Biotin-binding Sites** | 4 | 4 | 4 |
| **Isoelectric Point (pI)** | 10 | 6.8–7.5 | 6.3 |
| **Specificity** | Low | High | Highest |
| **Affinity for Biotin ($K_d$)** | $10^{-15}$ M | $10^{-15}$ M | $10^{-15}$ M |
| **Nonspecific Binding** | High | Low | Lowest |

## Immobilized Avidin Products

*Strong biotin interaction creates a nearly irreversible bond.*

Immobilized avidin can be used in a variety of applications for the affinity purification of biotinylated macromolecules. In one variation, an antibody that has an affinity for a particular antigen is labeled with biotin. Cells containing the antigen are lysed, then incubated with the biotinylated antibody to form a typical antigen/antibody complex. To isolate the antigen, the crude mixture is passed through an immobilized avidin or streptavidin column, which will bind the complex. After appropriate washes, the antigen can be eluted from the column with a low pH elution buffer. The biotinylated antibody is retained by the column.

### Applications:

- Binding biotinylated anti-transferrin for purifying transferrin from serum[1]
- Binding biotinylated peptides and elution with an SDS/urea solution[2]
- Hybridization of biotinylated RNA to its complementary DNA and binding to immobilized avidin, with subsequent elution of the single-stranded DNA[3]
- Purification of double-stranded DNA[4]

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **20219** | **Immobilized Avidin**<br>Support : Crosslinked 6% beaded agarose<br>Capacity: ≥20 µg biotin/ml gel | 5 ml |
| **20225** | **Immobilized Avidin**<br>Support and Capacity: Same as above | 5 x 5 ml |
| **20362** | **Immobilized Avidin Columns**<br>Support and Capacity: Same as above | 5 x 1 ml |


**References**

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## Immobilized Streptavidin Products

*Same high biotin-binding affinity as avidin with low nonspecific binding.*

### Applications:

- Purification of membrane antigens in conjunction with biotinylated monoclonal antibodies[1,2]
- Cell-surface labeling with biotinylation reagents, followed by precipitation with immobilized streptavidin[3]
- Purification of cell-surface glycoproteins using biotinylated Concanavalin A[4]
- Recovery of single-stranded DNA for dideoxy sequencing[5]

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **20347** | **Streptavidin Agarose Resin**<br>Support: Crosslinked 6% beaded agarose<br>Capacity: 1–3 mg biotinylated BSA/ml resin<br>15–28 µg biotin/ml resin | 2 ml |
| **20349** | **Streptavidin Agarose Resin**<br>Support and Capacity: Same as above | 5 ml |
| **20353** | **Streptavidin Agarose Resin**<br>Support and Capacity: Same as above | 10 ml |
| **20351** | **Streptavidin Agarose Columns**<br>Support and Capacity: Same as above | 5 x 1 ml |
| **53113** | **Streptavidin UltraLink Resin**<br>Support: UltraLink Biosupport<br>Capacity: ≥ 2 mg biotinylated BSA/ml resin<br>≥ 24 µg biotin/ml resin | 2 ml |
| **53114** | **Streptavidin UltraLink Resin**<br>Support and Capacity: Same as above | 5 ml |
| **53116** | **Streptavidin Plus UltraLink Resin**<br>Support: UltraLink Biosupport<br>Capacity: ≥ 4 mg biotinylated BSA/ml resin<br>≥ 48 µg biotin/ml resin | 2 ml |
| **53117** | **Streptavidin Plus UltraLink Resin**<br>Support and Capacity: Same as above | 5 ml |
| **20357** | **High Capacity Streptavidin Agarose Resin**<br>Support: Crosslinked 6% beaded agarose<br>Capacity: > 10 mg biotinylated BSA/ml of resin | 2 ml |
| **20359** | **High Capacity Streptavidin Agarose Resin**<br>Support and Capacity: Same as above | 5 ml |
| **20361** | **High Capacity Streptavidin Agarose Resin**<br>Support and Capacity: Same as above | 10 ml |
| **21344** | **MagnaBind Streptavidin Beads**<br>Support: 1–4 µm, iron oxide particles<br>Capacity: 2 µg biotin/ml beads | 5 ml |


**References**

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## Immobilized NeutrAvidin Products

*Less nonspecific binding produces cleaner results and better yields.*

When nonspecific binding is a problem in your application, Thermo Scientific Immobilized NeutrAvidin Products are superior alternatives to avidin or streptavidin. NeutrAvidin Biotin-Binding Protein is a modified avidin derivative that combines several key features to provide biotin-binding with exceptionally low nonspecific binding properties.

### Highlights:

- Carbohydrate-free – just like streptavidin, NeutrAvidin Biotin-Binding Protein has no carbohydrate, eliminating nonspecific binding problems due to sugars
- No interaction with cell surface molecules – absence of the Arg-Tyr-Asp sequence (present in streptavidin), which mimics the universal cell surface recognition sequence present in a variety of molecules, eliminates cross-reactivity of cell surface molecules
- Neutral pI – with a pI of 6.3, NeutrAvidin Protein has a pI that is closer to neutrality than avidin or streptavidin, eliminating electrostatic interaction that contributes to nonspecific binding

### Applications:

- Immunoprecipitation
- Purifying proteins that bind to biotinylated ligands
- Capturing biotinylated cell-surface proteins[1-3]
- Purifying biotinylated peptides[4]

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **29200** | **NeutrAvidin Agarose Resin**<br>Support: Crosslinked 6% beaded agarose<br>Capacity: > 20 µg or 80 nmol biotin/ml resin<br>(approx. 1–2 mg biotinylated BSA/ml resin) | 5 ml |
| **29201** | **NeutrAvidin Agarose Resin**<br>Support and Capacity: Same as above | 10 ml |
| **53150** | **NeutrAvidin UltraLink Resin**<br>Support: UltraLink Biosupport<br>Capacity: 12–20 µg biotin/ml gel | 5 ml |
| **53151** | **NeutrAvidin Plus UltraLink Resin**<br>Support: UltraLink Biosupport<br>Capacity: ≥ 30 µg biotin/ml gel | 5 ml |
| **29202** | **High Capacity NeutrAvidin Agarose Resin**<br>Support: Crosslinked 6% beaded agarose<br>Capacity: > 75 µg biotin/ml resin<br>> 8 mg biotinylated BSA/ml resin | 5 ml |
| **29204** | **High Capacity NeutrAvidin Agarose Resin**<br>Support and Capacity: Same as above | 10 ml |


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## Immobilized Monomeric Avidin and Kit

*Ideal affinity support for gentle, reversible binding of biotinylated proteins.*

To break the avidin-biotin interaction, 8 M guanidine•HCl at pH 1.5 or boiling in SDS-PAGE sample buffer is required. These elution methods may result in denaturation of the biotinylated protein and cause irreversible damage to the support. In addition, avidin or streptavidin will be irreversibly denatured and lose the ability to bind subsequent biotinylated samples.

When avidin is coupled to a solid support as the subunit monomer, the specificity for biotin is retained, but the affinity for biotin binding substantially decreases ($K_a \sim 10^8\ M^{-1}$). The Monomeric Avidin Agarose Resin and Kit can be used to bind biotinylated molecules, and the bound material can be competitively eluted using 2 mM biotin in phosphate-buffered saline (PBS). This technique provides the gentlest elution conditions without contamination of the avidin subunits or substantial loss of column-binding capacity.

### Highlights:

- Purifies biotinylated products under mild elution conditions
- Can be regenerated and reused at least 10 times
- Exhibits little nonspecific binding (3% or less)

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **20228** | **Monomeric Avidin Agarose Resin**<br>Support: Crosslinked 4% beaded agarose<br>Capacity: ≥ 1.2 mg biotinylated BSA/ml resin | 5 ml |
| **20267** | **Monomeric Avidin Agarose Resin**<br>Support and Capacity: Same as above | 10 ml |
| **20227** | **Monomeric Avidin Agarose Kit**<br>Support and Capacity: Same as above<br>Includes: 1 x 2 ml Column, Binding and Elution buffers | Kit |
| **53146** | **Immobilized Monomeric Avidin UltraLink Resin**<br>Support: UltraLink Biosupport<br>Capacity: ≥ 1.2 mg biotinylated BSA/ml resin | 5 ml |
| **29129** | **Biotin** | 1 g |


**References**

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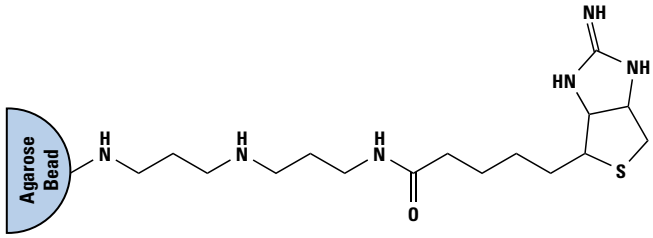
## Immobilized Iminobiotin and Biotin

*Iminobiotin offers mild dissociation conditions at pH 4.*




**Immobilized Iminobiotin**

Iminobiotin is the guanido analog of biotin. The dissociation constant of the avidin-iminobiotin complex is pH-dependent. At pH 9.5-11.0, the avidin-iminobiotin complex will bind tightly. At pH 4, the avidin-iminobiotin complex will dissociate. Because denaturing agents such as 8 M guanidine•HCl or 4 M urea are not used in the purification, an avidin conjugate has a better chance of maintaining its activity during purification.

Use immobilized D-Biotin as an "irreversible linkage" to bind streptavidin conjugates. The biotin-streptavidin interaction can withstand extremes in pH, salt and detergents.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **20221** | **Iminobiotin Agarose Resin**<br>Support: Crosslinked 6% beaded agarose<br>Spacer: Diaminodipropylamine<br>Capacity: ≥ 1 mg of avidin/ml resin | 5 ml |
| **20218** | **Biotin Agarose Resin**<br>Support: Pierce CDI Support<br>Spacer: Diaminodipropylamine<br>Capacity: ≥ 2 mg of avidin/ml resin | 5 ml |


**References**

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## MagnaBind Beads for Convenient Affinity Purification

*Thermo Scientific MagnaBind Magnetic Beads are ideal for solid-phase assays such as ELISAs, radioimmunoassays, cell separation and chemiluminescent immunoassays.*

### Magnetic separation offers the following advantages:

- Fast, easy separations
- Easily scalable
- Separation without the need for centrifugation
- Easily automated methodology

By using affinity chromatographic properties to purify specific molecules from a complex mixture, MagnaBind Beads offer rapid separations, high recovery and specificity. It is possible to isolate single populations of cells, specific proteins and nucleic acids with MagnaBind Technology. MagnaBind Streptavidin is perfect for tightly coupling your biotinylated protein or for capture of biotinylated oligonucleotides. MagnaBind Biotin Beads are available for purification of any biotin-binding molecule. Magnetic separation is a convenient, bench-top procedure for affinity separations of your molecules.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **21344** | **MagnaBind Streptavidin**<br>Support: 1-4 µm, iron oxide particles<br>Capacity: 2 µg biotin/ml beads | 5 ml |
| **21358** | **MagnaBind Magnet 96-Well Plate Separator** | 1 |
| **21359** | **MagnaBind Magnet Microcentrifuge Tubes** | 1 |
| **21357** | **MagnaBind Magnet for 1.5 ml Microcentrifuge Tubes** | 1 |

**References**

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## NeutrAvidin Coated Polystyrene Plates

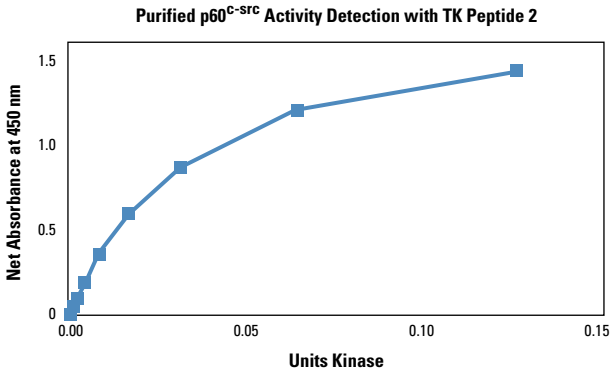
*The high affinity of avidin for biotin, without the nonspecific binding problems.*

### Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Lowest nonspecific binding properties of all biotin-binding proteins
- NeutrAvidin Biotin-Binding Protein has no carbohydrate and an isoelectric point of 6.3
- No denaturing of the protein component of a conjugate upon binding to the plate
- Ideal for binding small hydrophilic molecules (e.g., peptides) that typically exhibit poor binding directly to polystyrene
- Pre-blocked with your choice of Thermo Scientific Blocker BSA or SuperBlock Blocking Buffer
- Available in 96- and 384-well formats

### Characteristics of avidin-biotin proteins.

| Protein | Isoelectric Point | Contains Carbohydrate | Nonspecific Binding |
|---|---|---|---|
| **Avidin** | 10–10.5 | Yes | High |
| **Streptavidin** | 5.5 | No | Low |
| **NeutrAvidin Biotin-Binding Protein** | 6.3 | No | Ultralow |




**Biotinylated tyrosine kinase peptide 2 was added to Thermo Scientific NeutrAvidin Coated Plates and incubated for 30 minutes.** Wells were washed; samples containing p60c-src tyrosine kinase were added to phosphorylate the tyrosine residue on the peptide. Anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase was added. Tyrosine kinase activity was detected by Thermo Scientific 1-Step Turbo TMB Substrate. Kinase activity was quantitated by comparison with a standard curve generated using the phosphorylated form of the same peptide substrate.

**Reference**

Singh, Y., *et al.* (1999). *Infect. Immun.* **67,** 1853–1859.

## Ordering Information

| Product # | Coating | Plate Type | Blocking* | Binding Capacity† | Pkg. Size |
|---|---|---|---|---|---|
| **15129** | NeutrAvidin Protein, 100 µl | Clear, 96-Well | SuperBlock BB, 200 µl | ~ 15 pmol biotin/well | 5 plates |
| **15127** | NeutrAvidin Protein, 100 µl | Clear, 8-Well Strip | SuperBlock BB, 200 µl | ~ 15 pmol biotin/well | 5 plates |
| **15400** | NeutrAvidin Protein, 50 µl | Clear, 384-Well | SuperBlock BB, 100 µl | ~ 10 pmol biotin/well | 5 plates |
| **15116** | NeutrAvidin Protein, 100 µl | White, 96-Well | SuperBlock BB, 200 µl | ~ 15 pmol biotin/well | 5 plates |
| **15401** | NeutrAvidin Protein, 50 µl | White, 384-Well | SuperBlock BB, 100 µl | ~ 10 pmol biotin/well | 5 plates |
| **15117** | NeutrAvidin Protein, 100 µl | Black, 96-Well | SuperBlock BB, 200 µl | ~ 15 pmol biotin/well | 5 plates |
| **15402** | NeutrAvidin Protein, 50 µl | Black, 384-Well | SuperBlock BB, 100 µl | ~ 10 pmol biotin/well | 5 plates |
| **15123** | NeutrAvidin Protein, 200 µl | Clear, 96-Well | Blocker BSA, 300 µl | > 15 pmol biotin/well | 5 plates |
| **15128** | NeutrAvidin Protein, 200 µl | Clear, 8-Well Strip | Blocker BSA, 300 µl | > 15 pmol biotin/well | 5 plates |
| **15216** | NeutrAvidin Protein, 200 µl | White, 96-Well | Blocker BSA, 300 µl | > 15 pmol biotin/well | 5 plates |
| **15217** | NeutrAvidin Protein, 200 µl | Black, 96-Well | Blocker BSA, 300 µl | > 15 pmol biotin/well | 5 plates |
| **15115** | Biotin Binding Plate Sample Pack, one each of Product #s 15120, 15121, 15127, 15128 | | | | 4 plates |

** BB = Blocking Buffer*
*† Approximate values; plates tested for specific signal:noise and C.V.*

*All coated 96- and 384-well plates are available in bulk quantity with bulk packaging at a discounted price.*

*We can also custom-coat plates using a certain type of plate or a specific supplier's plate or coat with a specific surface chemistry that is not included in our standard product offering. Please contact our Large-Volume Custom Sales Team at 800-874-3723 or 815-968-0747 for more information. Outside the United States, contact your local branch office or distributor.*

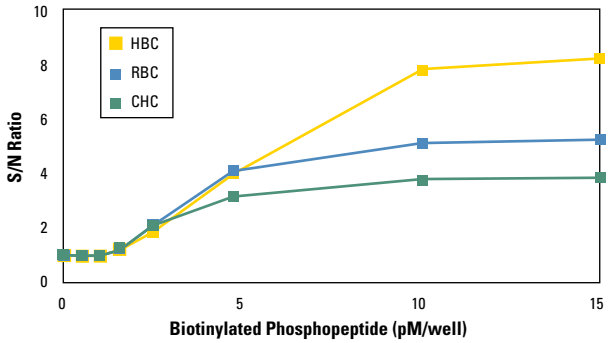
## NeutrAvidin High Binding Capacity (HBC) Coated Plates

*Unique technology for improved assay precision.*

We offer researchers a wide variety of avidin-biotin products, including our exclusive NeutrAvidin Coated Plates available in a high binding capacity (HBC) format. NeutrAvidin Protein is a deglycosylated form of avidin with a near-neutral pI that results in less nonspecific binding than that of streptavidin or avidin. Our patent-pending plate-coating technology offers a NeutrAvidin HBC Plate with a wider detection limit than our regular binding capacity plates. The standard curve exhibits greater linearity for detecting small biotinylated molecules such as peptides (see Figure) and oligonucleotides, resulting in greater assay precision. Try Thermo Scientific Pierce NeutrAvidin HBC Coated Plates for binding small biotinylated ligands and see the difference.

### Highlights:

- Unique plate-coating technology – results in high loading of NeutrAvidin Protein per well
- Improved sensitivity – less nonspecific binding for improved signal-to-noise ratios
- Broader dynamic range – extends the quantitative range so there's no need for dilutions
- Save time – pre-blocked plates to reduce the number of assay steps
- Flexible assay formats – coated plates offered in 96- and 384-well formats and in different colors




**Comparison of Thermo Scientific NeutrAvidin High Binding Capacity (HBC) Coated Plate, NeutrAvidin Regular Binding Capacity (RBC) Coated Plates and another supplier's Streptavidin Coated High Binding Capacity Plates (CHC).** Plates were incubated with various dilutions of biotinylated, phosphorylated peptide. After washing, the plates were incubated with mouse anti-phosphotyrosine antibody (1:1,000) and then detected using an anti-mouse-FITC conjugate (1:666). The Y-axis is described as the signal-to-noise (S/N) ratio.

### Ordering Information

| Product # | Coating | Plate Type | Blocking* | Binding Capacity† | Pkg. Size |
|---|---|---|---|---|---|
| 15507 | NeutrAvidin Protein, 100 µl | Clear, 96-Well | SuperBlock BB, 200 µl | ~ 60 pmol biotin/well | 5 plates |
| 15508 | NeutrAvidin Protein, 100 µl | Clear, 8-Well Strip | SuperBlock BB, 200 µl | ~ 60 pmol biotin/well | 5 plates |
| 15511 | NeutrAvidin Protein, 50 µl | Clear, 384-Well | SuperBlock BB, 100 µl | ~ 35 pmol biotin/well | 5 plates |
| 15509 | NeutrAvidin Protein, 100 µl | White, 96-Well | SuperBlock BB, 200 µl | ~ 60 pmol biotin/well | 5 plates |
| 15512 | NeutrAvidin Protein, 50 µl | White, 384-Well | SuperBlock BB, 100 µl | ~ 35 pmol biotin/well | 5 plates |
| 15510 | NeutrAvidin Protein, 100 µl | Black, 96-Well | SuperBlock BB, 200 µl | ~ 60 pmol biotin/well | 5 plates |
| 15513 | NeutrAvidin Protein, 50 µl | Black, 384-Well | SuperBlock BB, 100 µl | ~ 35 pmol biotin/well | 5 plates |

** BB = Blocking Buffer*
*† Approximate values; plates tested for specific signal:noise and C.V.*

## Pierce Streptavidin Coated Polystyrene Plates

*The specific binding affinity of streptavidin for biotin – in a microplate.*

### Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Low nonspecific binding
- No denaturing of the protein component of a conjugate upon binding
- Ideal for binding small biotinylated hydrophilic molecules (e.g., peptides) that typically exhibit poor binding to polystyrene
- Pre-blocked with your choice of Blocker BSA or SuperBlock® Blocking Buffer
- Available in clear, white and black plates in 12 × 8-well strip, 96-well and 384-well formats


**References**

Estrada, G., *et al.* (1996). *Mol. Cell Probes* **10,** 179–185.
Grobler, J.A. *et al.* (2002). *Proc. Nat. Acad. Sci., USA* **99,** 6661–6666.


### Ordering Information

| Product # | Coating | Plate Type | Blocking* | Binding Capacity† | Pkg. Size |
|---|---|---|---|---|---|
| **15124** | **Streptavidin, 100 µl** | Clear, 96-Well | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 plates |
| **15126** | **Streptavidin, 100 µl** | Clear, 96-Well | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 x 5 plates |
| **15120** | **Streptavidin, 100 µl** | Clear, 8-Well Strip | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 plates |
| **15122** | **Streptavidin, 100 µl** | Clear, 8-Well Strip | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 x 5 plates |
| **15405** | **Streptavidin, 50 µl** | Clear, 384-Well | SuperBlock BB, 100 µl | ~ 4 pmol biotin/well | 5 plates |
| **15118** | **Streptavidin, 100 µl** | White, 96-Well | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 plates |
| **15119** | **Streptavidin, 100 µl** | Black, 96-Well | SuperBlock BB, 200 µl | ~ 5 pmol biotin/well | 5 plates |
| **15407** | **Streptavidin, 50 µl** | Black, 384-Well | SuperBlock BB, 100 µl | ~ 4 pmol biotin/well | 5 plates |
| **15125** | **Streptavidin, 200 µl** | Clear, 96-Well | Blocker BSA, 300 µl | ~ 10 pmol biotin/well | 5 plates |
| **15121** | **Streptavidin, 200 µl** | Clear, 8-Well Strip | Blocker BSA, 300 µl | ~ 10 pmol biotin/well | 5 plates |
| **15218** | **Streptavidin, 200 µl** | White, 96-Well | Blocker BSA, 300 µl | ~ 10 pmol biotin/well | 5 plates |
| **15219** | **Streptavidin, 200 µl** | Black, 96-Well | Blocker BSA, 300 µl | ~ 10 pmol biotin/well | 5 plates |
| **15115** | **Biotin Binding Plate Sample Pack,** one each of Product #s 15120, 15121, 15127, 15128 | | | | 4 plates |

** BB = Blocking Buffer*
*† Approximate values; plates tested for specific signal:noise and C.V.*

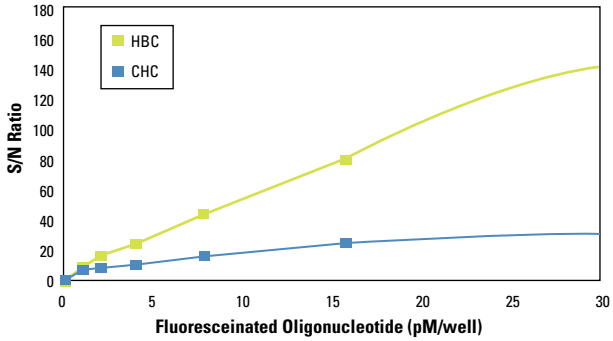
## Pierce Streptavidin HBC Coated Plates

*Take advantage of our technology that provides a broader dynamic range.*

Thermo Scientific Pierce Streptavidin High Binding Capacity (HBC) Coated Plates are designed for binding biotinylated oligonucleotides and peptides with higher binding efficiency than other commercially available plates. Our proprietary coating technology (patent pending) has created a streptavidin-coated plate with four- to five-times the binding capacity of other suppliers' plates. Using our Streptavidin HBC Plate can result in an assay with a broader dynamic range and better linearity, leading to improved assay precision (see Figure). Try our Streptavidin HBC Coated Plate and see what has been going undetected in your research.

### Highlights:

- Broader dynamic range – extends the quantitative range so there's no need for dilutions
- Better sensitivity – increased binding capacity allows direct detection of small ligands not observed with regular binding capacity plates
- Superior assay precision – standard curve demonstrates greater linearity
- Save time – pre-blocked to reduce number of assay steps
- Flexible assay formats – offered in 96- and 384-well formats and in different colors




**Comparison of Thermo Scientific Pierce Streptavidin High Binding Capacity (HBC) Coated Plate with another commercially available high binding capacity plate (CHC).** Plates were incubated with a biotinylated oligonucleotide, washed and probed with a complementary oligonucleotide labeled with fluorescein at various dilutions. The Y-axis is described as the signal-to-noise (S/N) ratio.

### Ordering Information

| Product # | Coating | Plate Type | Blocking* | Binding Capacity† | Pkg. Size |
|---|---|---|---|---|---|
| **15500** | Streptavidin, 100 µl | Clear, 96-Well | SuperBlock BB, 200 µl | ~ 125 pmol biotin/well | 5 plates |
| **15501** | Streptavidin, 100 µl | Clear, 8-Well Strip | SuperBlock BB, 200 µl | ~ 125 pmol biotin/well | 5 plates |
| **15504** | Streptavidin, 50 µl | Clear, 384-Well | SuperBlock BB, 100 µl | ~ 60 pmol biotin/well | 5 plates |
| **15502** | Streptavidin, 100 µl | White, 96-Well | SuperBlock BB, 200 µl | ~ 125 pmol biotin/well | 5 plates |
| **15505** | Streptavidin, 50 µl | White, 384-Well | SuperBlock BB, 100 µl | ~ 60 pmol biotin/well | 5 plates |
| **15503** | Streptavidin, 100 µl | Black, 96-Well | SuperBlock BB, 200 µl | ~ 125 pmol biotin/well | 5 plates |
| **15506** | Streptavidin, 50 µl | Black, 384-Well | SuperBlock BB, 100 µl | ~ 60 pmol biotin/well | 5 plates |

** BB = Blocking Buffer*
*† Approximate values; plates tested for specific signal:noise and C.V.*

## Thermo Scientific Avidin-based Conjugates

The noncovalent, high affinity of biotin for avidin ($K_a = 10^{15}\ M^{-1}$), with four biotin-binding sites per avidin molecule, allows more signal to be concentrated at a detection site. Below are just a few of the applications exploiting the avidin-biotin interaction.

- ELISA
- Immunohistochemical staining
- Western blotting
- DNA hybridization assays
- Immunoprecipitation
- Affinity chromatography
- Fluorescent activated cell sorting (FACS)

**Comparison of Thermo Scientific NeutrAvidin Biotin-Binding Protein, Avidin and Streptavidin.**

| Protein | MW | pI | Carbohydrate |
|---|---|---|---|
| **NeutrAvidin Biotin-Binding Protein** | 60 kDa | 6.3 | No |
| **Streptavidin** | 53 kDa | 6.8-7.5 | No |
| **Avidin** | 67 kDa | 10 | Yes |

## Thermo Scientific NeutrAvidin Products

*For ultralow nonspecific binding compared to avidin or streptavidin!*

Achieve better assay results with the low nonspecific binding properties of NeutrAvidin Protein. NeutrAvidin Protein is deglycosylated, so lectin binding is reduced to undetectable levels without losing biotin-binding affinity ($K_a = 10^{15}\ M^{-1}$). NeutrAvidin Protein neutral pI minimize nonspecific adsorption; lysine residues remain available for derivatization or conjugation through its amine-reactive chemistries. The specific activity for biotin-binding is approximately 14 µg/mg of protein.

### Highlights:

- Near-neutral pI (6.3) and no glycosylation, unlike avidin
- No RYD recognition sequence like streptavidin
- Generally lower nonspecific binding than avidin and streptavidin
- Much lower price than streptavidin

**References**

Hiller, Y., *et al.* (1987). *Biochem. J.* **248**, 167-171.
Unson, M.D., *et al.* (1999). *J. Clin. Microbiol.* **37**, 2153-2157.
Wojciechowski, M., *et al.* (1999). *Clin. Chem.* **45**, 1690-1693.
Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
Guo, Y., *et al.* (2001). *J. Biol. Chem.* **276**, 45791-45799.
Claypool, S.M., *et al.* (2002). *J. Biol. Chem.* **27**, 28038-28050.

### Ordering Information

| Product # | Description | Features | Pkg. Size |
|---|---|---|---|
| **31000** | NeutrAvidin Biotin-Binding Protein | • pI that has been reduced to a neutral state<br>• Deglycosylated, so lectin binding is reduced to undetectable levels<br>• Can be used as a biotin blocking agent in tissues for histochemistry<br>• 11-17 µg biotin bound/mg NeutrAvidin Protein | 10 mg |
| **31007** | Maleimide Activated NeutrAvidin Biotin-Binding Protein | • Prepare NeutrAvidin conjugates of proteins/peptides<br>• Reacts spontaneously with free sulfhydryls in the pH range of 6.5-7.5<br>• 4-8 moles maleimide/mole NeutrAvidin Protein | 5 mg |
| **31001** | NeutrAvidin Horseradish Peroxidase Conjugated | • Better signal-to-noise ratio in assay systems<br>• 1-2 moles HRP/mole NeutrAvidin Protein<br>• 3-8 µg biotin bound/mg conjugate | 2 mg |
| **31002** | NeutrAvidin Alkaline Phosphatase Conjugated | • Lower nonspecific binding than streptavidin conjugates<br>• Better signal-to-noise ratio in assay systems<br>• 3-8 µg biotin bound/mg conjugate | 2 mg |
| **31006** | NeutrAvidin Fluorescein Conjugated | • Fluorescent-labeled NeutrAvidin Biotin-Binding Protein<br>• Absorption: 490 nm; Emission 520 nm<br>• ≥ 2 moles fluorescein/mole NeutrAvidin Protein | 5 mg |
| **22831** | DyLight 405 NeutrAvidin | • Ex/Em: 400 nm and 420 nm | 1 mg |
| **22832** | DyLight 488 NeutrAvidin | • Ex/Em: 493 nm and 518 nm | 1 mg |
| **22837** | DyLight 549 NeutrAvidin | • Ex/Em: 562 nm and 576 nm | 1 mg |
| **22842** | DyLight 594 NeutrAvidin | • Ex/Em: 593 nm and 618 nm | 1 mg |
| **22844** | DyLight 633 NeutrAvidin | • Ex/Em: 638 nm and 658 nm | 1 mg |
| **22845** | DyLight 649 NeutrAvidin | • Ex/Em: 654 nm and 576 nm | 1 mg |
| **22848** | DyLight 680 NeutrAvidin | • Ex/Em: 682 nm and 715 nm | 1 mg |
| **22853** | DyLight 800 NeutrAvidin | • Ex/Em: 770 nm and 794 nm | 1 mg |

## Thermo Scientific Streptavidin Products

*Wide selection of conjugates for almost any biotin-based assay.*

Originally isolated from *Streptomyces avidinii*, streptavidin is a tetrameric biotin-binding protein that we produce and offer in recombinant form. Compared to the native protein, recombinant streptavidin is smaller that the native protein (MW 53kDa) and has a more neutral isoelectric point (pI 6.8-7.5). Streptavidin is carbohydrate-free and much less soluble in water than avidin, resulting in high binding affinity, capacity and specificity for biotinylated molecules.

### Ordering Information

| Product # | Description | Features | Applications | Pkg. Size |
|---|---|---|---|---|
| 21122 | Streptavidin | • Lyophilized, stable powder<br>• No carbohydrate<br>• Much less soluble in water than avidin<br>• 13-22 µg biotin bound/mg of protein<br>• Recombinant | • Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes<br>• Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin)<br>• Can be used with biotinylated enzymes (Product # 29339 or 29139) | 1 mg |
| 21125 | Streptavidin | | | 5 mg |
| 21120 | Hydrazide Activated | • Attaches streptavidin to oxidized carbohydrate residues on glycoproteins<br>• ≥ 4 moles hydrazide/mole streptavidin | • Used to create immunoassay reagents<br>• Localize glycoproteins on blot transfers, followed by detection with a biotinylated enzyme | 2 mg |
| 21102 | Maleimide Activated | • Attaches streptavidin to sulfhydryls | • Used to create immunoassay reagents | 1 mg |
| 21126 | Horseradish Peroxidase Conjugated | • 1-2 moles HRP/mole streptavidin | • Histochemistry | 1 mg |
| 21124 | Horseradish Peroxidase Conjugated | • ≥ 100 peroxidase units/mg conjugate | • Western blotting | 2 mg |
| 21127 | Horseradish Peroxidase Conjugated | • Lyophilized, stable powder<br>• 6-9 µg biotin bound/mg conjugate | • Conti, L.R., *et al.* (2001). *J. Biol. Chem.* 276, 41270-41278. | 5 mg |
| 21130 | High Sensitivity HRP Conjugated | • 1 mg/ml | • ELISA, Western, IHC | 0.5 ml |
| 21132 | High Sensitivity HRP Conjugated | • 1 mg/ml | • ELISA, Western, IHC | 5 ml |
| 21134 | High Sensitivity HRP Conjugated | • Pre-diluted (10 µg/ml) | • ELISA, Western, IHC | 1 mg |
| 21324 | Alkaline Phosphatase Conjugated | • ≥ 3 µg biotin bound/mg conjugate | • Histochemistry | 1 mg |
| 21323 | Alkaline Phosphatase Conjugated | • ≥ 100 phosphatase units/mg conjugate | • Western blotting<br>• Harriman, G.R., *et al.* (1999). *J. Immunol.* 162, 2521-2529. | 3 mg |
| 21224 | Fluorescein (FITC) Conjugated | • Ex/Em: 490 nm and 520 nm<br>• 3-5 moles FITC/mole streptavidin | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1 mg |
| 21724 | Rhodamine (TRITC) Conjugated | • Excitation: 515-520 nm and 550-555 nm<br>• Emission: 575 nm<br>• 1-3 moles TRITC/mole streptavidin | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1 mg |
| 21624 | Texas Red™ Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 595 nm and 615 nm | • Histochemical staining; can be used in double staining methods<br>• Fluorescence-activated cell sorting (FACS) | 1 mg |
| 21627 | R-Phycoerythrin Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 480, 545 and 565 nm and 578 nm | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1 ml |
| 21629 | Allophycocyanin Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 650 nm and 660 nm | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 0.5 ml |
| 21831 | DyLight 405 Streptavidin | • Ex/Em: 400 nm and 420 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21832 | DyLight 488 Streptavidin | • Ex/Em: 493 nm and 518 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21837 | DyLight 549 Streptavidin | • Ex/Em: 562 nm and 576 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21842 | DyLight 594 Streptavidin | • Ex/Em: 593 nm and 618 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21844 | DyLight 633 Streptavidin | • Ex/Em: 638 nm and 658 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21845 | DyLight 649 Streptavidin | • Ex/Em: 654 nm and 673 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21848 | DyLight 680 Streptavidin | • Ex/Em: 682 nm and 715 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21850 | DyLight 750 Streptavidin | • Ex/Em: 752 nm and 778 nm | • ELISA, Western, FACS, IHC | 1 mg |
| 21851 | DyLight 800 Streptavidin | • Ex/Em: 770 nm and 794 nm | • ELISA, Western, FACS, IHC | 1 mg |

## Thermo Scientific Avidin Products

*Convenient conjugates for assay detection.*

Avidin is a tetrameric glycoprotein (MW 67kDa) purified from chicken egg white. The highly specific interaction of avidin with biotin makes it a useful tool in designing nonradioactive detection systems. The extraordinary affinity of avidin for biotin ($K_a = 10^{15}\ M^{-1}$) allows biotin-labeled molecules to be detected with excellent sensitivity and specificity.

Avidin is more soluble than streptavidin and has an isoelectric point (pI) of 10.5. It is also more economical than streptavidin, and is commonly used in signal amplification systems such as the ABC system.


**References**

Chaiet, I. and Wolf, F.J. (1964). *Arch. Biochem. Biophys.* **106**, 1-5.
Savage, M.D., *et al.* (1992). *Avidin-Biotin Chemistry: A Handbook.* Rockford, Illinois: Pierce Chemical Company.
Wilchek, M. and Bayer, E.A. (1983). *Anal. Biochem.* **171**, 1-32.
Gitlin, G., *et al.* (1987). *Biochem. J.* **242**, 923-926.
Bruch, R.C. and White, III, H.B. (1982). *Biochemistry* **21**, 5334-5341.
Zuk, P.A. and Elferink, L.A. (2000). *J. Biol. Chem.* **275**, 26754-26764.


### Ordering Information

| Product # | Description | Features | Applications | Pkg. Size |
|---|---|---|---|---|
| **21121** | **Avidin** | • Hen egg white glycoprotein, affinity-purified, salt-free, lyophilized powder<br>• 11-14 µg biotin bound/mg avidin<br>• Isoelectric point of 10-10.5<br>• Stable over a wide range of pH and temperatures | • Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes<br>• Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin) | 10 mg |
| **21128** | **Avidin** | | | 20 mg |
| **21123** | **Horseradish Peroxidase Conjugated** | • Purified using special affinity techniques to eliminate nucleic acids<br>• 1-2 moles HRP/mole avidin<br>• 5-10 µg biotin bound/mg protein<br>• ≥ 80 peroxidase units/mg protein | • Use in immunohistochemistry where endogenous phosphatase is a problem<br>• Western blotting | 2 mg |
| **29994** | **Horseradish Peroxidase Conjugated** | | | 5 mg |
| **21321** | **Alkaline Phosphatase Conjugated** | • Homogeneous by SDS-PAGE<br>• Purified using special affinity techniques to eliminate nucleic acids<br>• ~1 mole alkaline phosphatase/mole avidin<br>• One unit = 1.0 micromole of *p*-nitrophenol liberated from *p*-nitrophenylphosphate per minute at 37°C, pH 9.5 | • Use for immunohistochemistry where high levels of endogenous peroxidase is a problem<br>• Western blotting<br>• ELISA | 100 units |
| **21221** | **Fluorescein (FITC) Conjugated** | • Fluorescent-labeled avidin<br>• Ex/Em: 490 nm and 520 nm<br>• No free fluorescein<br>• ~3.5 moles fluorescein/mole avidin | • Fluorescence-activated cell sorting (FACS)<br>• Histochemical staining | 5 mg |
| **21021** | **R-Phycoerythrin Conjugated** | • Fluorescent-labeled avidin<br>• Ex/Em: 450-570 nm and 574 nm | • Fluorescence-activated cell sorting (FACS)<br>• Histochemical staining | 1 mg |

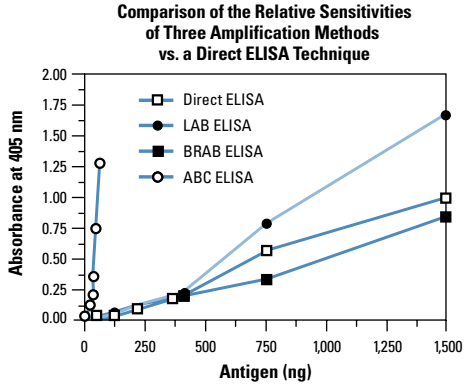
## ABC Staining Kits

Thermo Scientific Pierce ABC Staining Kits are highly sensitive, have rapid avidin-biotin interactions and produce very low background staining. Highly diluted primary antibodies can be used with Thermo Scientific Pierce Staining Kits, producing comparable stain intensity to other methods that require higher concentrations of antibody.

Two types of enzymatic ABC staining kits, alkaline phosphatase and horseradish peroxidase, are available. The kits can come with or without (standard kit) a biotinylated secondary antibody.

Kits with a secondary antibody are selected according to the species of primary antibody to be used. For example, if the primary antibody (IgG) is produced in mice, the kit selected to detect this antibody should be the ABC Mouse IgG Kit. To create a sensitive detection system, you need your specific primary antibody, an ABC Mouse Kit with biotinylated antibody, and an enzyme substrate.

The standard kit includes only the avidin and biotinylated enzyme and is useful if an ABC Kit is not available for your specific species of primary antibody, or if the primary antibody is already labeled with biotin. The biotinylated antibody can then be used, along with a blocking agent, and used with a standard kit.




### The ABC Kits contain:

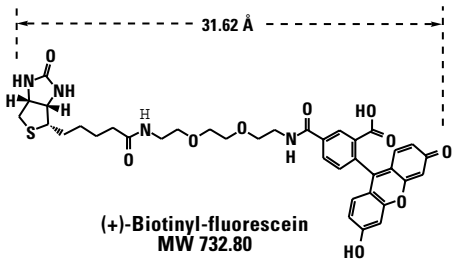
- 3 ml of the appropriate blocking serum
- 1 ml of the biotinylated affinity-purified secondary antibody
- 2 ml each of the avidin and biotinylated enzyme reagents

Our Ultra-Sensitive ABC Peroxidase Staining Kits are more sensitive than the ABC Peroxidase Staining Kits, without exhibiting increased background staining. These kits supply the extra sensitivity needed for localizing antigens present in very small quantities. An expensive primary antibody may be diluted approximately five-fold higher than it could be with the ABC Peroxidase Kit, while producing equal staining intensity.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 32052 | **Ultra-Sensitive ABC Peroxidase Mouse IgG Staining Kit**<br>Includes: Biotinylated Anti-Mouse IgG Antibody Blocking Buffer Avidin Biotinylated HRP | Kit |
| 32054 | **Ultra-Sensitive ABC Peroxidase Rabbit IgG Staining Kit**<br>Includes: Biotinylated Anti-Rabbit IgG Antibody Blocking Buffer Avidin Biotinylated HRP | Kit |
| 32020 | **Standard Peroxidase Staining Kit**<br>Includes: Avidin Biotinylated HRP | Kit |
| 32050 | **Ultra-Sensitive ABC Standard Peroxidase Staining Kit**<br>Includes: Avidin Biotinylated HRP | Kit |

## Biotin Fluorescein




**Notes:**
- Water-soluble
- Perfect for diagnostic applications
- Absorbance: 492 nm
- Color: greenish-yellow

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **22030** | **Biotin Fluorescein** | 5 mg |

### Ordering Information

| Product # | Notes | Description | Pkg. Size |
|---|---|---|---|
| **29130** | • Commonly used as a molecular weight marker on SDS-PAGE and in gel permeation<br>• Frequently used as a blocking agent in many immunological techniques<br>• Useful as a control in ELISA, immunoblotting and immunohistochemical studies<br>• Supplied as a lyophilized powder with 8-12 moles of biotin per mole of BSA | **Biotinylated Bovine Serum Albumin (BSA)** | 25 mg |
| **29139** | • An extremely pure reagent with virtually all enzyme activity retained after biotinylation<br>• Offers excellent sensitivity and is recommended for use in sandwich techniques that utilize Thermo Scientific Pierce Avidin, Streptavidin or NeutrAvidin Protein<br>• Unit is defined as amount of Biotinylated-HRP required to form 1 µmole of purpurogallin from pyrogallol in 20 seconds at 20°C<br>• Reconstituted with distilled water to 1 mM citrate, pH 6 | **Biotinylated Horseradish Peroxidase** | 5 mg |
| **29339** | • Alkaline phosphatase has been isolated from calf intestine<br>• Biotinylation of this enzyme does not alter its high specific activity<br>• Can be used as a secondary marker in immunohistochemical staining<br>• One unit equals the amount of protein needed to hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at 25°C in the following buffer: 0.1 M glycine, 1.0 mM $ZnCl_2$, 1.0 mM $MgCl_2$, 6 mM PNPP, pH 10.4 | **Biotinylated Alkaline Phosphatase** | 1 mg |
| **29939** | • Used with ONPG for histochemical and immunoblotting applications<br>• Can be used as a detection reagent<br>• One unit = 1 µmole *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed per minute at 37°C, pH 7.3 | **Biotinylated β-Galactosidase** | 100 units |
| **29988** | • Useful as a substitute for biotinylated secondary antibodies<br>• Can detect or locate immunoglobulins on cell surface or in tissue<br>• Recombinant form of Protein G, altered to remove the albumin binding sites to reduce nonspecific binding | **Biotinylated Protein G** | 0.5 mg |
| **29989** | • Useful as a substitute for biotinylated secondary antibodies<br>• Can detect or locate immunoglobulins on cell surface or in tissue cell surface or in tissue | **Biotinylated Protein A** | 1 mg |
| **29997** | • Binds to the $V_L$ region of kappa light chains (human I, III, IV and Mouse I) without interfering with antigen-binding sites<br>• Binds to all classes of IgG (e.g., IgG, IgM, IgA, IgE and IgD)<br>• Does not bind bovine, goat or sheep immunoglobulins<br>• Binds single-chain variable fragments (ScFv) | **Biotinylated Protein L** | .5 mg |
| **31826** | • Prepared with highly purified IgG<br>• Detect low concentrations of Fc receptors or anti-immunoglobulin antibodies on cells or in tissue<br>• Use with a NeutrAvidin Conjugate to detect receptors for IgG<br>• Supplied in PBS or HBS (10 mM HEPES, 0.15 M NaCl, pH 7.8) containing 0.04% sodium azide | **Biotinylated Rabbit IgG** | 5 mg |
| **29129** | • Free biotin is used in the elution buffer to purify biotinylated compounds from monomeric avidin columns<br>• Can be used as a reference standard for the HABA dye assay when measuring degree of biotinylation | **D-Biotin** | 1 g |

### Biotinylated Secondary Antibodies

We offer more than 40 biotin-labeled secondary antibodies. View a complete selection guide for secondary antibodies in the Products section of our web site at www.thermo.com/pierce

## Example Protocols for Biotinylation

Additional protocols can be found in individual product instruction booklets that can be downloaded from our website.

The molar ratio of biotinylation reagent to protein is one of the most important parameters to consider when biotin-labeling a protein. This ratio will determine the degree of labeling that is achieved. A high degree of labeling can ensure that each protein molecule contains at least one biotin moiety. However, a low degree of biotinylation ensures minimal modification of the native protein, resulting in maximum retention of biological activity. For large proteins such as antibodies, a modification level of three to five biotins/protein is generally optimal, while small proteins may require slightly less modification. The optimal biotinylation level for any protein can only be determined experimentally.

### Biotinylating IgG with EZ-Link Sulfo-NHS-Biotin Reagents

Preparing stock solutions of any NHS-esters of biotin with the intent of long-term storage is not recommended because hydrolysis occurs very quickly in solution. The container of biotinylation reagent must be brought to room temperature prior to opening, then the proper amount of biotinylation reagent is weighed, dissolved and used immediately. Even those NHS-esters that require organic solvents should be made up immediately before use as these solvents (i.e., DMSO and DMF) are hygroscopic and water absorbed from the air results in hydrolysis of the reagent.

These conditions generally result in an immunoglobulin with a modification level of approximately three to five biotins per molecule of IgG. The molar ratio of Sulfo-NHS-Biotin to protein may be adjusted to achieve a particular outcome.

### Materials required:

- Biotinylation reagent such as Sulfo-NHS-LC-Biotin (Product # 21335)
- PBS (0.1 M phosphate, 0.15 M NaCl; pH 7.2; or Product # 28372) or other non-amine containing buffer at pH 7.0-8.5 (recommend pH 7.5-8.0)
- Device for removal of unreacted biotin such as Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kits for dialysis of 0.5-3.0 ml samples (Product # 66382) or Thermo Scientific Zeba Spin Desalting Columns, 5K MWCO, for desalting of samples up to 2 ml (Product # 89891)

### Procedure:

1. Allow the biotinylation reagent to warm completely to room temperature before opening the vial.
2. Dissolve 2-10 mg of IgG in 1 ml PBS.
3. Immediately before use, make a 10 mM stock solution of Sulfo-NHS-LC-Biotin.
4. Add sufficient volume of the concentrated Sulfo-NHS-LC-Biotin to give a 12-fold molar excess of biotin to a 10 mg/ml IgG solution or a 20-fold molar excess of biotin to a 2 mg/ml IgG solution (See Table 1 for examples).
5. Place the reaction mixture on ice and incubate for two hours. Alternatively, incubate at room temperature for 30 minutes.
6. Use dialysis or gel filtration to remove the unreacted Sulfo-NHS-LC-Biotin. See instructions provided with preferred buffer exchange product.
7. Store the biotinylated protein under the same condition that is optimal for the non-biotinylated protein.

**Amount of biotinylation reagent to add to a 1 ml protein sample.**

| Protein | MW of Protein | mg Protein/ml | mMole Protein/ml | Fold Molar Excess of Biotin | mM Biotin Reagent to Add | Volume of 10 mM Biotin Reagent to add |
|---|---|---|---|---|---|---|
| Protein A | 42,000 | 10 | $2.4 \times 10^{-4}$ | 12 | $2.9 \times 10^{-3}$ | 290 µl |
| | | 2 | $4.8 \times 10^{-5}$ | 20 | $9.5 \times 10^{-4}$ | 95 µl |
| BSA | 68,000 | 10 | $1.5 \times 10^{-4}$ | 12 | $1.8 \times 10^{-3}$ | 180 µl |
| | | 2 | $3.0 \times 10^{-5}$ | 20 | $5.9 \times 10^{-4}$ | 59 µl |
| IgG | 150,000 | 10 | $6.7 \times 10^{-5}$ | 12 | $8.0 \times 10^{-4}$ | 80 µl |
| | | 2 | $1.3 \times 10^{-5}$ | 20 | $2.7 \times 10^{-4}$ | 27 µl |

## Troubleshooting guide for biotinylation with NHS-esters.

| Problem | Cause | Solution |
|---|---|---|
| Poor biotinylation | No amines available on molecule of interest | • Choose biotinylation reagent that targets different group.<br>• Convert sulfhydryl to amine using Aminoethyl-8 (Product # 23010). |
| | Inappropriate choice of buffer | • Choose non-amine-containing buffer at pH 7-9 (hydrolysis is very rapid at higher pH). |
| | Hydrolysis of biotinylation reagent | • Allow reagent to come to RT before opening.<br>• Make up fresh biotinylation reagent. |
| | Incomplete removal of primary amines | • Use biotinylation reagent immediately.<br>• Dialyze or desalt thoroughly into non-amine-containing buffer at pH 7-9. |
| Protein loses function | Over-biotinylation | • Reduce molar excess of biotinylation reagent.<br>• Reduce time or temperature for biotinylation.<br>• Choose biotinylation reagent that targets different groups. |

## Biotinylating Cell Surface Proteins

Selective labeling of proteins located at the cell surface is often desirable in the study of integral membrane proteins. Cell surface biotinylation has emerged as an important tool for studying the expression and regulation of receptors and transporters, differentiation of plasma membrane proteins from those localized to organelle membranes, and distribution of membrane proteins in polarized epithelial cells. The specificity of Sulfo-NHS-esters of biotin for cell surface labeling has been demonstrated in these applications.[2,3] A variety of similar protocols have been used successfully on a wide range of cell types.[1,2,3,4,5,6,7] Because these molecules dissolve readily in polar solutions and are charged by the sodium sulfoxide group on the succinimidyl ring, they cannot permeate the cell membrane. As long as the cell is intact, only primary amines exposed on the surface are biotinylated. When internal biotinylation is desired, our NHS-LC-Biotin (Product # 21336), or other non-water-soluble analogs, can be used.

Following cell surface biotinylation, it is often necessary to isolate the biotinylated proteins. This is best accomplished using immobilized NeutrAvidin Biotin-Binding Protein, when harsh elution conditions are appropriate, or Monomeric Avidin, when a milder elution is indicated for the recovery of functional protein molecules. Several example protocols for affinity purification of biotinylated molecules are given in the following pages of this booklet.

### Materials required:

- Sulfo-NHS-LC-Biotin (Product # 21335) or other Sulfo-NHS-ester biotinylation reagent
- PBS (0.1 M phosphate, 0.15 M NaCl; pH 7.2; or Product # 28372)


**References**

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Yang, B., *et al.* (2009). *FASEB J.* **23**, 503-512.
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### Procedure:

1. Wash cells three times with ice-cold PBS, pH 8.0, to remove any contaminating proteins.
2. Suspend the cells at a concentration of ~25 x $10^6$ cells/ml in PBS, pH 8.0.
   **Note:** Other cell concentrations can be used. The concentration of biotinylation reagent can be scaled up or down accordingly, based on cell size, type, etc.
3. Add 1 mg of Sulfo-NHS-LC-Biotin per ml of reaction volume.
4. Incubate at room temperature for 30 minutes.
   **Note:** Performing this incubation at 4°C may reduce active internalization of the biotinylation reagent.
5. Wash cells three times with ice-cold PBS + 100 mM glycine to quench any remaining biotinylation reagent.

The cell surface proteins are now biotinylated on exposed lysine residues.

Thermo Scientific Pierce Cell Surface Protein Isolation Kit is a convenient way to biotin-label and purify mammalian cell-surface proteins. This kit efficiently labels proteins with accessible lysine residues and sufficient extracellular exposure. The kit includes all the reagents necessary for labeling, cell lysis and purification of cell-surface proteins.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **89881** | **Cell Surface Protein Isolation Kit** | Kit |
| | Includes: EZ-Link Sulfo-NHS-SS-Biotin | |
| | Quenching Solution | 8 x 12mg vials |
| | Lysis Buffer | 16 ml |
| | Immobilized NeutrAvidin Gel | 4.5 ml |
| | Wash Buffer | 2.25 ml gel slurry |
| | Column Accessory Pack | 34 ml |
| | No-Weigh Dithiothreitol (DTT) | 8 spin columns |
| | BupH Phosphate Buffered Saline | 8 x 7.7 mg |
| | BupH Tris Buffered Saline | 2 packs |
| | | 1 pack |

# Example Protocols for Biotinylation

## One-Step Biotinylation and Dialysis in a Slide-A-Lyzer Cassette

Small-volume samples have the inherent problem of sample loss due to handling. This can be an especially important complication if several processing procedures are required. The use of a Slide-A-Lyzer Dialysis Cassette (SAL) can prevent sample loss because more than one reaction can be performed in a single container, thus eliminating transfer steps. The following is a sample protocol for biotinylating a small amount of protein. A similar method can be used for other reactions inside the SAL.

### Materials required:

- Sulfo-NHS-LC-Biotin (Product # 21335) or other Sulfo-NHS-ester biotinylation reagent
- PBS (0.1 M phosphate, 0.15 M NaCl; pH 7.2; or Product # 28372)
- Slide-A-Lyzer Kit (Product # 66382, includes cassettes, syringes, needles and buoys)
- Protein solution (This protocol is written for 1 ml of a 2-10 mg/ml solution. The choice of SAL and amount of biotinylation reagent can be adjusted to suit other protein amounts.)

### Procedure:

1. Allow the biotinylation reagent to warm completely to room temperature before opening the vial.
2. Immediately before use, make a 10 mM solution of Sulfo-NHS-LC-Biotin.
3. Add biotinylation reagent directly to the protein in PBS. Add sufficient volume of the 10 mM Sulfo-NHS-LC-Biotin to give a 12-fold molar excess of biotin to a 10 mg/ml IgG solution or a 20-fold molar excess of biotin to a 2 mg/ml IgG solution (See Table 1 for examples).

   **Note:** Buffers other than PBS can be used, provided they are at pH 7.0-8.5 and do not contain primary amines.
4. Inject the mixture into a port the SAL, leaving an air bubble for mixing.
5. Tape the SAL onto a shaking platform and incubate for 30 minutes to 1 hour at room temperature or 2 hours at 4°C, shaking so that the bubble can be seen "mixing" the reagents.
6. Remove SAL from the shaking platform, insert the needle into another port and withdraw the air bubble.
7. Insert the SAL into the groove in the SAL buoy. Place the cassette and buoy in PBS (or other appropriate buffer) and dialyze.

**Note:** The use of 3 x 500 ml of PBS for 2 hours each is sufficient for dialysis. Less time may be sufficient for some systems. Following dialysis, the sample can be concentrated within the same SAL using Slide-A-Lyzer Concentrating Solution (Product # 66526).

8. Insert the needle into any port, withdraw the dialyzed sample and transfer to an appropriate storage container such as a microcentrifuge tube.

**Biotinylation troubleshooting guide.**

| Problem | Cause | Solution |
|---|---|---|
| No Avidin binding | Insufficient biotinylation | • Increase concentration of biotin reagent in coupling reaction.<br>• Increase incubation time of coupling reaction and maintain concentration of biotin reagent.<br>• Do not use Tris or glycine buffers when coupling amine-reactive labeling reagents. |
| | Biotin spacer arm is too short | • Use long chain (LC or LC-LC) analog of biotinylation reagent. |
| Loss of protein activity | Over-biotinylation | • Reduce concentration of biotin reagent in protein activity coupling reaction.<br>• Reduce incubation time of coupling reaction and maintain concentration of biotin reagent. |
| | Steric hindrance | • Direct biotinylation toward different residues (i.e., switch fromamine-reactive to sulfhydryl-reactive reagent). |

# Example Protocols for Affinity Purification Based on Avidin-Biotin Binding

## Introduction

Affinity purification makes use of a specific binding interaction that occurs between molecules and that is used extensively for the isolation of biological molecules. A single pass through an affinity column can achieve a 1,000- to 10,000-fold purification of ligand from a crude mixture. From a single affinity purification step, it is possible to isolate a compound in a form pure enough to obtain a single band upon SDS-PAGE analysis.

In affinity purification, a ligand is immobilized to a solid support. Once immobilized, it specifically binds its partner under mild buffer conditions (often physiologic conditions such as phosphate buffered saline). After binding to the partner molecule, the support is washed with additional buffer to remove unbound components of the sample. An elution buffer is added. The elution buffer disrupts the interaction between the ligand and its binding partner by pH extremes (low or high), high salt, presence of detergents, chaotropic agents that unfold one or both of the molecules, or the removal of some factor required for the pair to bind. Once released, the binding partner can be recovered from the support using additional elution buffer. The buffer can then be exchanged by dialysis or desalting into a more suitable buffer for storage or downstream analysis.

Any molecule that has an interacting partner can be attached to a support and used for affinity purification. The extraordinary affinity of avidin for biotin allows biotin-containing molecules to be discretely bound to immobilized avidin. Once biotin is attached to a molecule, the molecule can be affinity purified using an immobilized version of any biotin-binding protein. Alternatively, a biotinylated molecule can be immobilized through interaction with a biotin-binding protein, then used to affinity purify other molecules that specifically interact with it.

## Affinity Purification of Biotinylated Molecules (Column Format)

### Materials required:

- Immobilized Avidin, Streptavidin or NeutrAvidin Biotin-Binding Protein.
- Biotinylated sample in solution (~3 mg biotinylated protein/ml immobilized biotin-binding protein).
- Binding buffer: Phosphate Buffered Saline (0.1 M phosphate, 0.15 M NaCl; pH 7.2; Product # 28372). To reduce nonspecific binding, add 1% of a detergent such as NP-40 to the binding buffer.
- Elution buffer: 8 M guanidine•HCl, pH 1.5 (Product # 24115).
- Columns: Product # 89896 for resin volumes of 2 ml or less or Product # 89898 for resin volumes of 2-10 ml.

### Procedure:

1. Pack gel into the column.
2. Equilibrate the column with three column volumes of binding buffer.
3. Add biotinylated sample to the column and allow sample to enter the gel bed. Sequentially replace the bottom and top caps and incubate at room temperature for 30 minutes.

**Note:** If the sample is large enough that the entire amount cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Add more sample and incubate. Do not exceed the binding capacity of the gel.

4. Wash the column with 10 column volumes of binding buffer.
5. Elute the biotinylated molecules with 5-10 column volumes of the elution buffer. Collect the eluate in 0.5-1 ml fractions. Monitor protein content by measuring the absorbance of each fraction at 280 nm.
6. Desalt or dialyze the eluted fractions of interest to put them into a more suitable buffer.

# Example Protocols for Affinity Purification Based on Avidin-Biotin Binding

## Affinity Purification Using a Biotinylated Antibody (Column Format)

**Materials required:**

- Immobilized Avidin, Streptavidin or NeutrAvidin Biotin-Binding Protein.
- Biotinylated antibody in solution (~3 mg biotinylated antibody/ml immobilized biotin-binding protein).
- Binding buffer: Phosphate Buffered Saline (0.1 M phosphate, 0.15 M NaCl; pH 7.2; Product # 28372). To reduce nonspecific binding, add 1% of a detergent such as NP-40.
- Elution buffer: IgG Elution Buffer (Product # 21004), Gentle Ag/Ab Elution Buffer (Product # 21027) or 0.1 M glycine•HCl, pH 2.8.
- Columns: Product # 89896 for resin volumes of 2 ml or less or Product # 89898 for resin volumes of 2-10 ml.

**Procedure:**

1. Pack gel into the column.
2. Equilibrate the column with three column volumes of binding buffer.
3. Add biotinylated antibody to the column and allow solution to enter the gel bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes.

   **Note:** If the sample is large enough that the entire amount cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Add more antibody and incubate. Do not exceed the binding capacity of the gel.
4. Wash the column with 10 column volumes of binding buffer.
5. Add antigen-containing sample to the column and allow it to enter the gel bed. Replace the bottom and top caps sequentially and incubate at room temperature for 1-2 hours.
6. Wash the column with 10 column volumes of binding buffer.
7. Elute the antigen with 5-10 column volumes elution buffer. Collect the eluate in 0.5-1 ml fractions. If using IgG Elution Buffer or 0.1 M glycine•HCl, pH 2.8, immediately adjust the pH by the adding 1/10 volume of 1 M phosphate, pH 7.5. Monitor protein content by measuring the absorbance of each fraction at 280 nm.

   **Note:** If using Gentle Ag/Ab Elution Buffer, wash column with three column volumes of Tris Buffered Saline before antigen elution. The Gentle Elution Buffer is not compatible with phosphate buffers.
8. Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.

   **Note:** The column with the immobilized biotinylated antibody may be reused to purify more antigen. Wash column with 10 column volumes of binding buffer, add a solution of 0.02% sodium azide and store at 4°C.

## Immunoprecipitation Using a Biotinylated Antibody (Batch Format)

**Materials required:**

- Immobilized Avidin, Streptavidin or NeutrAvidin Biotin-Binding Protein.
- Biotinylated sample in solution (~3 mg biotinylated protein/ml immobilized biotin- binding protein).
- Binding buffer: Phosphate Buffered Saline (0.1 M phosphate, 0.15 M NaCl; pH 7.2; Product # 28372). To reduce nonspecific binding, add 1% of a detergent such as NP-40.
- Elution buffer: Pierce IgG Elution Buffer (Product # 21004), ImmunoPure Gentle Ag/Ab Elution Buffer (Product # 21027) or 0.1 M glycine•HCl, pH 2.8.
- Microcentrifuge tubes and Spin Columns (Product # 69725).

**Procedure:**

1. In a microcentrifuge tube, solubilize antigen in 50 µl of binding buffer and add the biotinylated antibody. Adjust the volume of the sample to 0.2 ml with binding buffer.
2. Incubate the sample overnight at 4°C.
3. Mix the immobilized avidin to ensure an even suspension. Add the appropriate amount of immobilized avidin to the tube containing the antigen/biotinylated antibody mixture.
4. Incubate the sample with mixing for 1 hour at room temperature or 4°C.
5. Wash the avidin-bound complex with 0.5-1.0 ml of binding buffer and centrifuge for 1-2 minutes at approximately 2,500 x g. Remove the supernatant. Repeat this wash procedure at least four times and remove the final wash.
6. Add elution buffer to the gel to recover the bound antigen. If using our IgG Elution Buffer or 0.1 M glycine•HCl, pH 2.8, remove the liquid and immediately adjust the pH by adding a suitable more concentrated buffer such as 1 M Tris, pH 7.5 (100 µl of this buffer to 1 ml of the sample is sufficient).

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