

Well Insert Holder in Deep Petri Dish - AVP015



Product Information Leaflet

Technology for Routine Three
Dimensional (3D) Cell Culture

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alvetex 

Technology for Routine Three Dimensional (3D) Cell Culture

Well Insert Holder in Deep Petri Dish AVP015

Product Information Leaflet

Supplied: Single well insert holder capable of housing three well inserts (not supplied) in a deep Petri dish.

- Reinnervate's custom-made "Well Insert Holder in a Deep Petri Dish" (AVP015) is designed to hold three 6 or 12-well inserts containing alvetex[®] discs. The Petri dish itself is not tissue culture treated.
- Alvetex[®] is a 200 μm thick cross-linked polystyrene membrane with ~90% porosity and a mean void size of 35-40 μm to create a 3D culture.
- The product has been terminally sterilised by gamma irradiation and remains sterile until opened.

FOR IN VITRO RESEARCH USE ONLY

**NOT FOR CLINICAL, DIAGNOSTIC,
OR THERAPEUTIC PROCEDURES**

NOT FOR USE IN HUMANS

PATENT PENDING

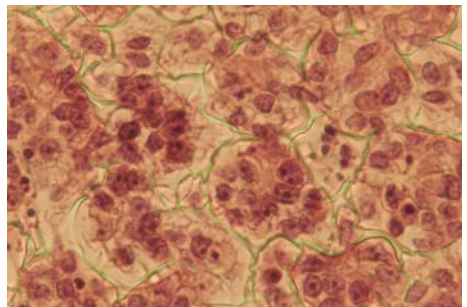
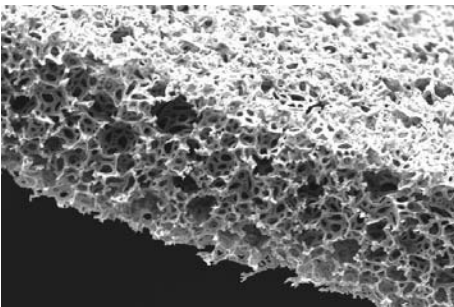
1.0. What is 3D cell culture?

3D cell culture is about creating suitable surroundings for optimal cell growth, differentiation and function by:

- Allowing individual cells to maintain their normal 3D shape and structure with minimal exogenous support and interference,
- Encouraging cells to form complex interactions with adjacent cells and receive and transmit signals,
- Enabling a more natural environment to foster the creation of native architecture found in tissue structures,
- Reducing stress and artificial responses as a result of cell adaptation to flat, 2D growth surfaces.

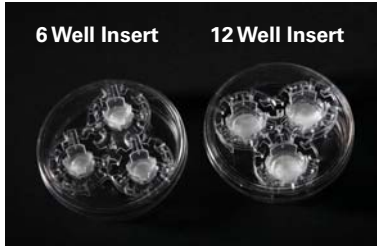
2.0 What is alvetex®?

Alvetex® is a highly porous, cross-linked polystyrene scaffold, which has been sectioned into 200 µm thick membranes (below left). The resulting material is inert and does not degrade during normal use. It has been adapted to fit a variety of conventional cell culture plastic ware formats. Alvetex® provides a suitable, 3D structure in which cells can proliferate, migrate, differentiate and function in an appropriate niche environment. Cells maintain a 3D shape and form close interactions with adjacent cells (below right, TERA2.cl.SP12 cells maintained for 12 days). The material is compatible with a broad range of standard molecular, cellular and histological techniques (visit www.reinnervate.com for further details).



3.0 Alvetex[®] well insert formats and their use in the well insert holder

The presentation of alvetex[®] in well insert format is versatile, enabling long term 3D culture as cells can receive nutrients from media above and below the membrane, sustaining optimal 3D cell growth.



AVP005-3 (left) and AVP004-3 (right) in well insert holder systems in deep Petri dishes.

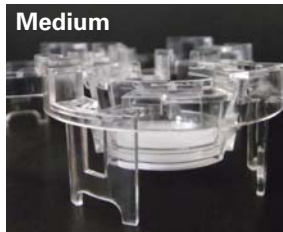
Currently there are two well insert sizes available: AVP004-3 (22mm diameter) and AVP005-3 (15mm diameter). The former is designed to fit a 6 well plate and the latter both 6 and 12-well plates; refer to respective product information leaflets for their use in these systems. Both of these well inserts will also fit Reinnervate's custom-made well insert holder system (AVP015).

The deep Petri dish enables users to grow their 3D cultures in larger volumes of media compared to an ordinary multiwell plate. Up to 95ml of media can be used in the deep Petri dish and is therefore capable of sustaining long term 3D culture experiments (3-4 weeks) and reducing the frequency of medium exchanges.

If required, a magnetic stirrer bar can be placed in the bottom of the dish to circulate media and facilitate exchange.



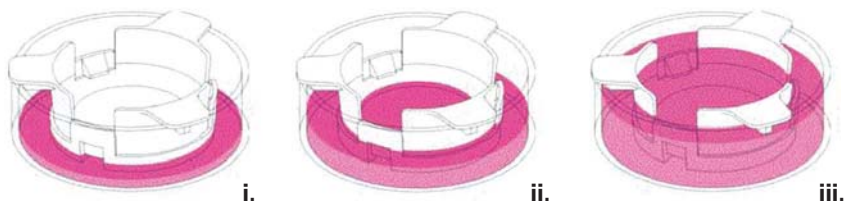
The well insert can be positioned at three different levels in the insert holder: high, medium and low:



This feature allows cultures to be raised to the air liquid interface by moving the insert to a different level within the same holder.

Positioning the well inserts at different levels may be used to conserve expensive media or allow for increasing media volumes for demanding cell types over the course of a long term experiment.

Additionally, cultures can be fed from below the well insert only (i.), media from above and below separately (ii.) and media interconnected (iii.) through the windows at the side of the well inserts:



Recommended volumes for AVP004-3 (6-well insert):

Well insert setting within holder	Feeding regime		
	Below only (i.)	Above and below separately (ii.)	Above and below interconnected (iii.)
Low	20ml \pm 1ml	40ml \pm 3ml	70ml \pm 5ml
Medium	34ml \pm 2ml	50ml \pm 3ml	80ml \pm 3ml
High	48ml \pm 2ml	70ml \pm 5ml	92ml \pm 3ml

Recommended volumes for AVP005-3 (12-well insert):

Well insert setting within holder	Feeding regime		
	Below only (i.)	Above and below separately (ii.)	Above and below interconnected (iii.)
Low	20ml \pm 1ml	40ml \pm 3ml	72ml \pm 5ml
Medium	34ml \pm 2ml	52ml \pm 3ml	82ml \pm 3ml
High	48ml \pm 2ml	70ml \pm 5ml	92ml \pm 3ml

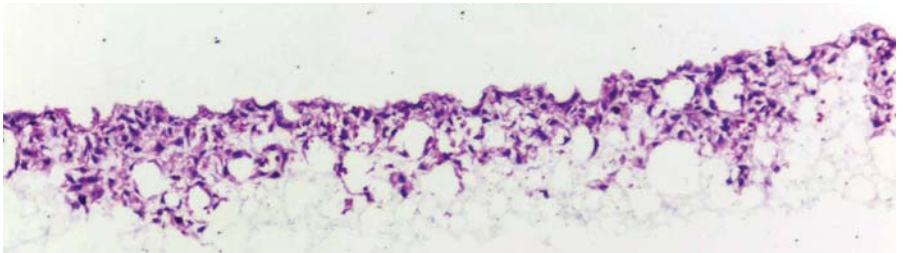
3.1 General precautions for handling alvetex[®] 6- and 12-well insert formats (AVP004-3 and AVP005-3) for use in well insert holder in deep Petri dish (AVP015)

- All procedures concerning handling of alvetex[®] well inserts should be performed wearing gloves according to standard aseptic methods required for cell culture in a Class I/II cabinet according to relevant product information leaflets.
- Dry alvetex[®] is reasonably fragile with a wafer-like consistency; however the rehydrated disc is more robust. Therefore handle the material carefully when performing any manipulation including cell seeding, media changes, transferring the discs for analysis, fixing and embedding for histology, etc. When using forceps and pipettes, exercise care whilst manipulating the well inserts as the discs can be easily damaged.
- When dispensing liquids (e.g. 70% EtOH, PBS and medium) over alvetex[®], place the end of the pipette tip towards the wall of the culture vessel (either by going through the window of the well insert or beside it). Let the liquid rise gently to touch the base of the well insert and if required dispense the rest of the solution into the well insert to prevent it from floating.
- The easiest way to treat alvetex[®] is by dipping the well insert into a beaker containing 70% EtOH before placing it into the well insert holder at the chosen setting inside the Petri dish. Alternatively EtOH treatment can be performed in situ by adding sufficient 70% EtOH to the well so that the level of the liquid rises above that of the membrane (this will be dependant on the setting used). Ensure that before seeding, the well insert is washed twice with an appropriate medium (ideally the same as used for the generation of the cell suspension).
- Seed cells on the middle of the disc without touching the membrane itself. Refer to relevant well insert product information leaflets for cell seeding volumes and conditions.
- Similarly to 2D culture, if using serum-free medium, consider the use of coating agents to enhance cell attachment.
- Prior to cell seeding, alvetex[®] can be also pre-coated with standard cell culture reagents such as collagen, fibronectin, laminin, poly-D/L-lysine, poly-L-ornithine and matrigel to encourage cell adhesion, differentiation and optimise function. Perform this step after the EtOH treatment followed by an appropriate buffer wash step instead of medium.
- The well insert holder system also allows for the 3D co-culture of more than one cell type by seeding different cells in one or two of the well inserts within the same Petri dish. 3D co-cultures can also be also setup within the same well insert. Alternatively, a support cell line can be

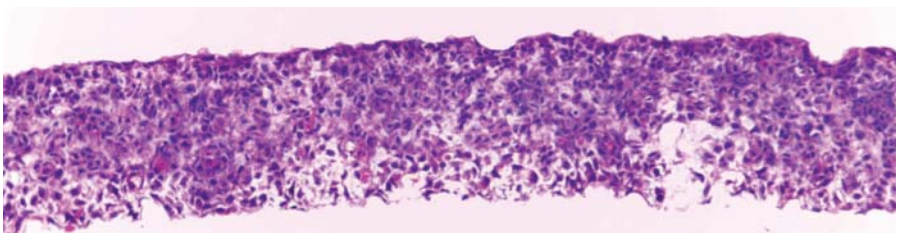
cultured at the base of the Petri dish in 2D and another in 3D in the well inserts. As the Petri dishes are untreated, coat with poly-L-lysine or similar to facilitate cell attachment. Alternatively the well insert holder will fit into deep Petri dishes with approximate dimensions of 86mm (internal diameter) x 25mm (height). Ensure that suitable media is chosen that will simultaneously support the growth of both cell types cultured.

- Each alvetex[®] disc is held in place to the bottom of the body component by a semi-continuous base. If required, this base can be unclipped at the end of an experiment using forceps, releasing the disc for further analysis. Alternatively, the discs can be cut out using a scalpel with a size 11 blade.

3.2 Comparison of cell growth pattern on alvetex[®] 12-well insert format (in 6-well plate versus well holder in Petri dish)



HaCaTs grown on alvetex[®] 12-well insert (AVP005-3) format in a 6-well plate.



HaCaTs grown on alvetex[®] 12-well insert (AVP005-3) format in "well insert holder in a deep Petri Dish" (AVP015).

Human keratinocyte cell line HaCaT was seeded (0.5x10⁶ cells in 50 µl per well) on EtOH-treated alvetex[®] scaffolds (AVP005-3) in 6-well plates and well insert holder in Petri dishes and maintained for 10 days by complete media exchange every second day. [Complete medium consisted of: DMEM, 10% FBS, 2mM L-glutamine and 100U/ml Penicillin & Streptomycin]. After preserving in Bouin's fixative the discs were paraffin embedded, sectioned (10 µl) and counterstained with Haematoxylin and Eosin. Note significantly more proliferation and cell invasion of the cultures grown in well-insert holder system where more nutrients are available.

3.3 Applications of other alvetex[®] formats

It is recommended that alvetex[®] in 12-well plates (AVP002) is used for short term experiments (7-10 days), with easy access to the cells resident in the upper layers of the membrane for applications such as transfection. Cultures grown in 6- (AVP004-3) and 12-well inserts (AVP005-3) on the other hand are suitable for long-term experiments (1-3 weeks), where maximum cell penetration and generation of high yields are required. Use the "well insert holder in a deep Petri Dish" (AVP015) for the prolonged culture of highly proliferative and demanding cell types in order to reduce the need for frequent media changes.

For further information see technical support at

www.reinnervate.com

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