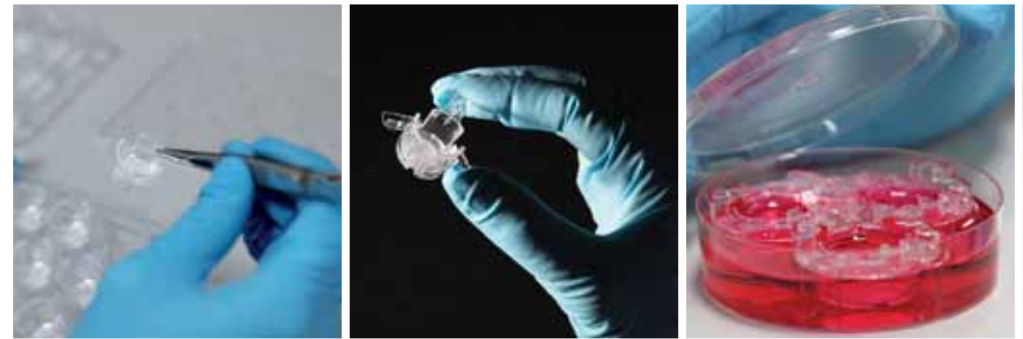


12-Well Insert Containing  
Alvetex® Polystyrene Scaffold - AVP005-3



Product Information Leaflet

Technology for Routine Three  
Dimensional (3D) Cell Culture

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**alvetex** 

# Technology for Routine Three Dimensional (3D) Cell Culture

## 12-Well Insert Containing alvetex® Polystyrene Scaffold AVP005-3

### Product Information Leaflet

**Supplied:** 3 x individually sealed 12-well inserts containing alvetex® discs for routine 3D cell culture. *Well insert holder not supplied.*

- Alvetex® is a 200 µm thick cross-linked polystyrene membrane with ~90% porosity and a mean void size of 35-40 µm.
- The well insert is designed to fit into most 6-well plates or Reinnervate's custom-made Well Insert Holder in Deep Petri Dish (AVP015). Snapping the extended wings will also enable it to fit into a 12-well plate.
- This format is untreated and requires an ethanol (EtOH) wash prior to use to render it hydrophilic.
- 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**

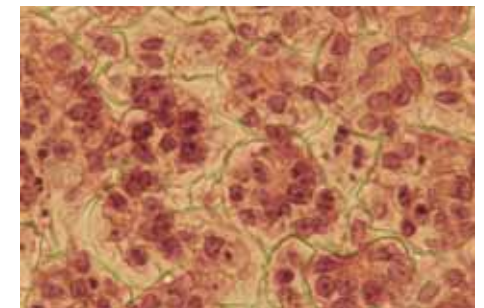
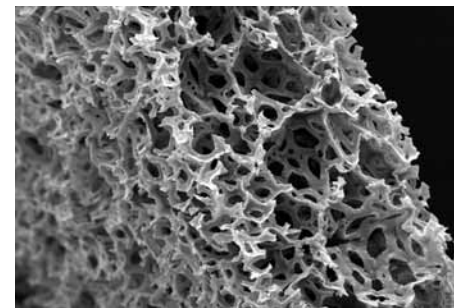
## 1.0 What is 3D cell culture?

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](http://www.reinnervate.com) for further details).



### 3.0 alvetex® 12-well insert format

The presentation of alvetex® in a 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. The well insert system can also be used for the creation of co-cultures.



The unit comprises a 200 µm thick alvetex® disc (15mm diameter) which is clipped 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.

The 12-well insert has extended wings that can snap back enabling it to fit into a 12 well plate. Unmodified, the 12-well insert fits into most 6-well plates or a Well Insert Holder in Deep Petri Dish (AVP015).

#### 3.1. Handling alvetex® 12-well insert format

- All procedures concerning the 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.
- When dry alvetex® is reasonably fragile with a wafer-like consistency; however once rehydrated the disc becomes much 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 during 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.*
- Seed cells on the middle of the disc without touching the membrane itself.

### 3.2. Use of 12-well inserts in 6-well plates

#### 3.2.1. Preparing non-treated alvetex® (12-well inserts) for first use and cell seeding in 6-well plates

- Open the required number of blister packs carefully and pick up the well insert using forceps.
- Immersion in 70% EtOH will instantly pre-treat alvetex® in preparation for incubation in aqueous solutions (e.g. PBS, culture medium). This can be done by dipping the well insert into a beaker containing 70% EtOH before placing it into the chosen holder vessel.
- Alternatively EtOH treatment can be performed *in situ*, once the well insert is positioned in the plate. Add sufficient 70% EtOH to the well so that the level of the liquid rises above that of the membrane (approximately 5ml per well).
- Carefully aspirate to waste and immediately wash alvetex® in an appropriate medium (7ml/well) for ~1 min.
- Aspirate and replace with final wash medium (use same type of medium as for cell seeding). The scaffold is now ready for cell seeding: aspirate medium just before application of cells. If preparation of cell suspension is delayed, incubate plate with medium at 37°C with 5%CO<sub>2</sub> until further use.
- 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 also be 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.

#### 3.2.2. Optimisation of seeding and 3D cell culture using the alvetex® 12-well insert format in 6-well plate

3D cell culture is different to conventional 2D cell culture and as such requires optimisation according to cell type:

- For most applications initial cell seeding densities of 0.25-1.0x10<sup>6</sup> cells in 50-75 µl per disc are often suitable. Seeding in a low volume enables cells to attach predominantly to the disc and avoids cell loss on other surfaces.

- When inoculating, aspirate washing media thoroughly from the plate and carefully dispense cells on the middle of the discs without touching the membrane. Replace lid and incubate in a humidified incubator at 37°C with 5% CO<sub>2</sub> for ~3 hours to facilitate cell attachment.
- After this time gently flood the wells with media by dispensing 3.0-10.5ml of medium per well: Fill up the wells carefully beside the insert, so the medium comes up from the bottom to gently contact the cellularised alvetex<sup>®</sup> disc and gradually floods the insert itself.
- The volume of medium required will depend on user requirements:



Medium from below only: for cells grown in 3D at air-liquid interface (3.5±0.5ml/well)



Media from above and below: for routine 3D growth of cells with lower-average metabolic activity/ proliferation rate (7±1ml/well) OR for experiments where cells are incubated with test substrate in top chamber only for permeability investigations



Media interconnected: for routine 3D growth of cells with high metabolic activity/ proliferation rate (10±0.5ml/well)

- In 3D cell culture there will be more cells growing per unit volume of medium. Therefore, users must refresh media more frequently typically every 2±1days, however this will also depend on the population doubling rate, nutrient demands of the cell type cultured and the volume of medium used as described above.
- If any sign of cell attachment and growth is evident on the bottom of the 6-well plate, transfer the well inserts into a new 6-well plate, re-feed and then incubate as usual.

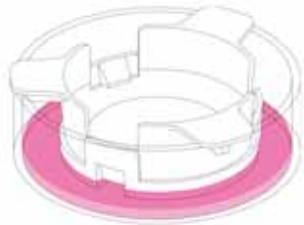
### 3.3. Use of 12-well inserts in 12-well plates

#### 3.3.1. Preparing non-treated alvetex<sup>®</sup> (12-well inserts) for first use and cell seeding in 12-well plates

- Open the required number of blister packs carefully and pick up the well insert using forceps.
- Immersion in 70% EtOH will instantly pre-treat alvetex<sup>®</sup> in preparation for incubation in aqueous solutions (e.g. PBS, culture medium). This can be done by dipping the well insert into a beaker containing 70% EtOH before placing it into the chosen holder vessel.
- Alternatively EtOH treatment can be performed *in situ*, once the well insert is positioned in the plate. Add sufficient 70% EtOH to the well so that the level of the liquid rises above that of the membrane (approximately 2ml per well).
- Carefully aspirate to waste and immediately wash alvetex<sup>®</sup> in an appropriate medium (2.5ml/well) for ~1 min.
- Aspirate and replace with final wash medium (use same type of medium as for cell seeding). The scaffold is now ready for cell seeding: aspirate medium just before application of cells. If preparation of cell suspension is delayed, incubate plate with medium at 37°C with 5%CO<sub>2</sub> until further use.
- Similarly to 2D culture, if using serum-free medium, consider the use of coating agents to enhance cell attachment.
- Prior to cell seeding, alvetex<sup>®</sup> 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.

### 3.3.2. Optimisation of seeding and 3D cell culture using the alvetex® 12-well insert format in 12-well plate

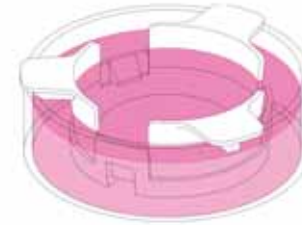
- 3D cell culture is different to conventional 2D and as such requires optimisation according to cell type:
- For most applications initial cell seeding densities of  $0.25\text{-}1.0 \times 10^6$  cells in  $50\text{-}75 \mu\text{l}$  per disc are often suitable. Seeding in a low volume enables cells to attach predominantly to the disc and avoids cell loss on other surfaces.
- When inoculating, aspirate washing media thoroughly from the plate and carefully dispense cells on the middle of the discs without touching the membrane. Replace lid and incubate in a humidified incubator at  $37^\circ\text{C}$  with  $5\% \text{CO}_2$  for  $\sim 3$  hours to facilitate cell attachment.
- After this time gently flood the wells with media by dispensing  $1.4\text{-}4.0\text{ml}$  of medium per well: Fill up the wells carefully beside the insert, so the medium comes up from the bottom to gently contact the cellularised alvetex® disc and gradually floods the insert itself.
- The volume of medium required will depend on user requirements:



**Medium from below only:** for cells grown in 3D at air-liquid interface ( $1.6 \pm 0.2\text{ml/well}$ )



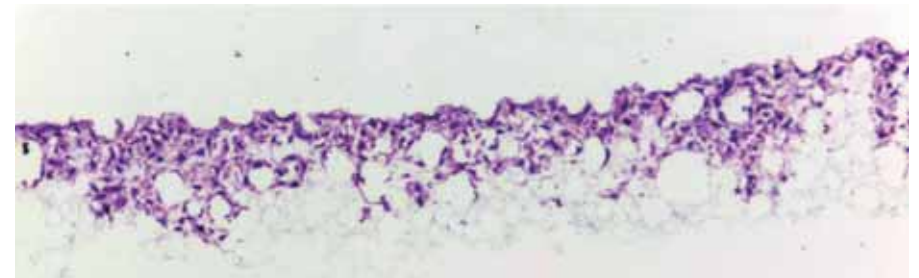
**Media from above and below:** for routine 3D growth of cells with lower-average metabolic activity/ proliferation rate ( $2.4 \pm 0.2\text{ml/well}$ ) OR for experiments where cells are incubated with test substrate in top chamber only for permeability investigations



**Media interconnected:** for routine 3D growth of cells with high metabolic activity/ proliferation rate ( $3.8 \pm 0.2\text{ml/well}$ )

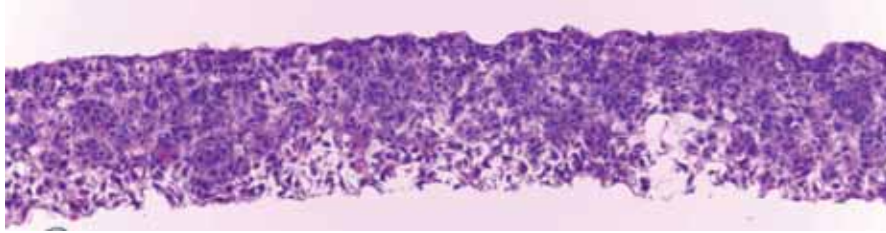
- In 3D cell culture there will be more cells growing per unit volume of medium. Therefore, users must refresh media more frequently typically every  $2 \pm 1$  days, however this will also depend on the population doubling rate and nutrient demands of the cell type cultured and the volume of medium used as described above.
- If any sign of cell attachment and growth is evident on the bottom of the 12-well plate, transfer the well inserts into a new 12-well plate, re-feed and then incubate as usual.

### 3.4. 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 Deep Petri Dish (AVP015).**

Human keratinocyte cell line HaCaT was seeded ( $0.5 \times 10^6$  cells in 50  $\mu$ l per well) on EtOH-treated alvetex® scaffolds (AVP005) 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 Bouins fixative the discs were paraffin embedded, sectioned (10  $\mu$ m) 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.

### 3.5. 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 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 on using both the 6 and 12-well inserts with the Well Insert Holder in Deep Petri Dish please refer to the AVP015 Product Information Sheet.

For further information see technical support at

[www.reinnervate.com](http://www.reinnervate.com)

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