



Product Information Leaflet

Technology for Routine Three
Dimensional (3D) Cell Culture

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Technology for Routine Three Dimensional (3D) Cell Culture

Alvetex[®] 12-Well Plate Format AVP002

Product Information Leaflet

Supplied: a single 12-well plate containing 12x loose alvetex[®] discs with clips.

- Alvetex[®] is a 200 µm thick cross-linked polystyrene membrane with ~90% porosity and a mean void size of 35-40 µm.
- The scaffold has been adapted to fit into the base of a conventional 12-well culture 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.

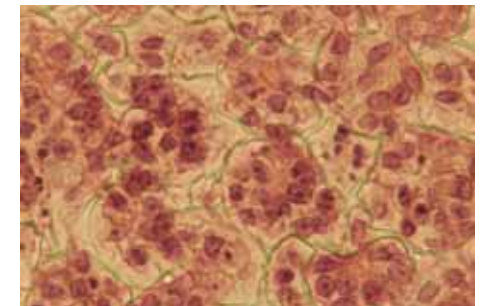
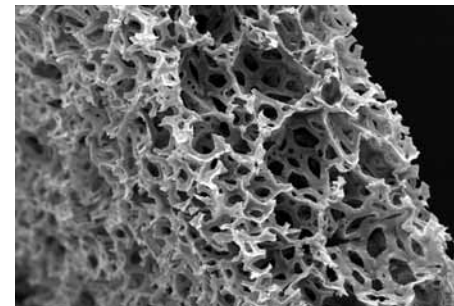
1.0 What is 3D cell culture?

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).



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

NOT FOR USE IN HUMANS

PATENT PENDING

3.0 Alvetex® 12-well plate format

Presentation of alvetex® in a 12-well plate comprises a single loose disc and clip per well. The clip holds alvetex® in position during transit and use. The clip is made from polystyrene, it is sterile and inert, and can easily be removed to release the alvetex® disc.

The 12-well plate format is a simple presentation of alvetex® technology. It is primarily suitable for short term culture experiments where the medium is replaced every 1-2 days (see below). Alternative presentations of alvetex® are available in the form of the well insert, which are capable of supporting prolonged cell growth and also allow for the creation of co-culture systems.

3.1 Handling alvetex® 12-well plate format

All procedures concerning the handling of alvetex® 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 discs become much more robust. Therefore handle the material carefully when performing any manipulation including media changes, transferring the discs for analysis, fixing and embedding for histology, etc. When using forceps, exercise care as manipulating the scaffold can damage its structure. Try to handle the alvetex® discs around the edges only.
- 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 avoid touching the scaffold. Retain cylindrical clip in place.
- Seed cells on the middle of the disc without touching the membrane itself.

3.1.1 Preparing non-treated alvetex® (in 12-well plates) for first use and cell seeding

- Open the 12-well plate carefully to ensure that the clips holding the alvetex® discs are not displaced.
- Add approximately 2ml of 70% EtOH to each well to pre-treat the alvetex® disc in preparation for incubation in aqueous solutions (e.g. PBS, culture medium).
- Carefully aspirate the EtOH solution and immediately wash alvetex® disc in ~2-3 ml appropriate medium for ~1 min.
- Carefully aspirate medium wash and replace with final wash medium (use same type of medium as for cell seeding). The alvetex® disc 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% CO₂ 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 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.

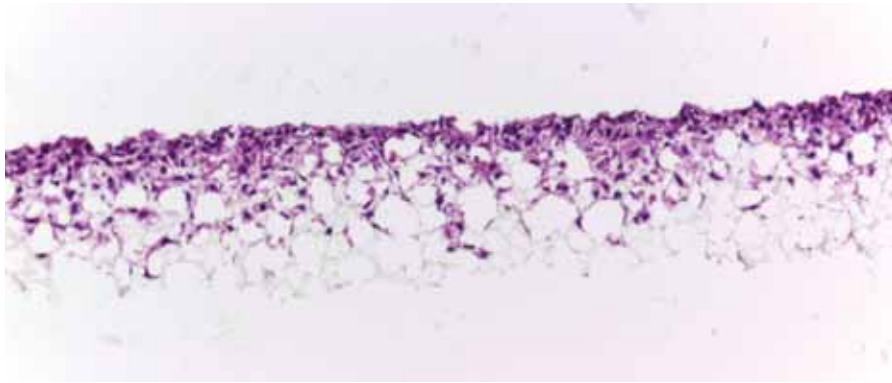
3.1.2 Optimisation of seeding and 3D cell culture using the alvetex® 12-well format

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 0.5-2.0x10⁶ cells in 100-150 µl per disc are recommended. Seeding in a low volume enables cells to attach predominantly to the alvetex® disc and avoids cell loss on other surfaces.
- When inoculating, aspirate washing medium thoroughly from the plate and carefully dispense cells on the middle of the discs. Replace lid and incubate in a humidified incubator at 37°C with 5% CO₂ for ~3 hours to facilitate cell attachment.
- After this time gently flood the wells with medium by dispensing up to 4ml of medium per well.
- With 3D cell culture there will be many more cells growing per unit volume of medium. Therefore, users must refresh media more frequently; ideally once a day, however this will also depend on the population doubling rate and nutrient demands of the cell type cultured.

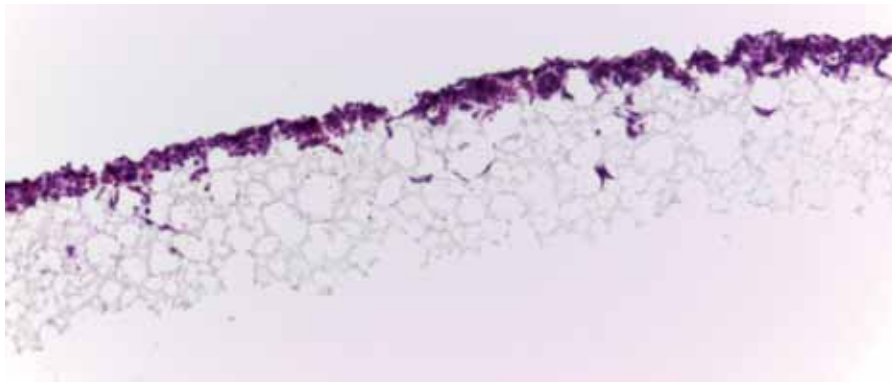
3.2 Examples of cell growth pattern on alvetex® (12-well plate format):

3.2.1. HaCaTs:



Human keratinocyte cell line HaCaT was seeded (0.5×10^6 cells in 150 μ l per well) on EtOH-treated and complete medium washed alvetex® discs. Cultures were incubated for three hours before flooding with further medium and maintained for 7 days. [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 μ m) and counterstained with Haematoxylin and Eosin demonstrating significant cell invasion into the matrix.

3.2.1. HepG2:



Human liver cell line HepG2 was seeded (2×10^6 cells in 150 μ l per well) on EtOH-treated and complete media washed scaffolds. Cultures were incubated for three hours before flooding with further media and maintained for 7 days. [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 μ m) and counterstained with Haematoxylin and Eosin to reveal cells resident in top 25% of the scaffold.

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), enabling easy access to the cells resident in the upper layers of the membrane for applications such as transfection. Cultures grown in 6-well inserts (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

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