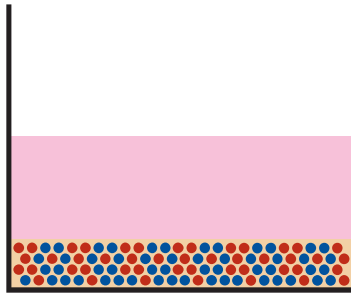


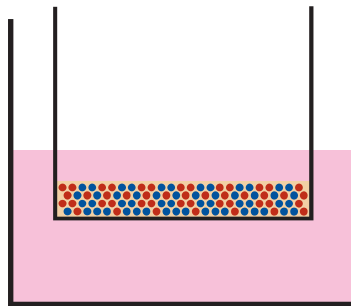
## Using alvetex® to create co-culturing experiments

### 1 Assembly option 3D / 3D co-culture in multi-well plate or well insert

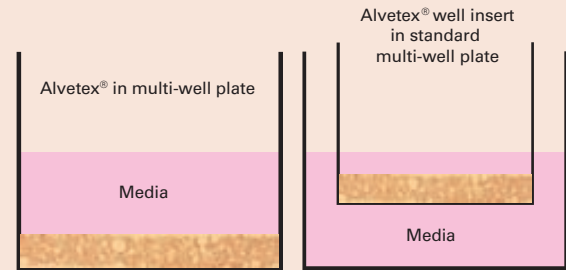


**Description**  
Different cell types cultured together within the same scaffold

**Application**  
Emulate the structure of a tissue comprised of more than one cell type



### Key to Image Parts:



 Cell type A growing in 3D within alvetex®

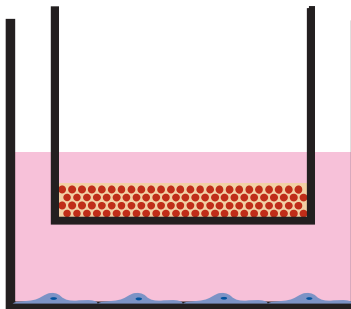
 Cell type B growing in 3D within alvetex®

 Mix of cells A&B in 3D

 Alvetex®

 Cells growing in 2D

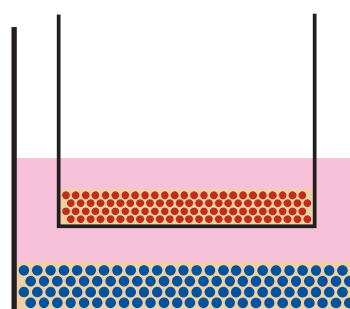
### 2 Assembly option 3D / 2D co-culture in multi-well plate and well insert combined



**Description**  
Two independent cell cultures one in 2D and one in 3D. Contact is via medium – communication via paracrine factors

**Application**  
Approach used to study the secretion of factors and signalling molecules

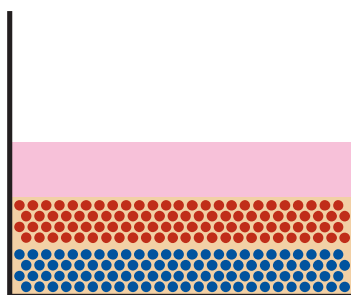
### 3 Assembly option 3D co-culture in multi-well plate and well insert combined



**Description**  
Two independent 3D cultures. Contact is via medium only and inter-culture communication via paracrine factors

**Application**  
Approach used to study the secretion of factors and signalling molecules

### 4 Assembly option 3D / 3D co-culture in multi-well plate

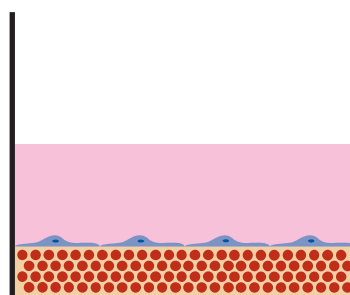


**Description**  
Two 3D cultures in direct contact

**Application**

- Study the direct interaction of cells in contact with one another
- To establish layers of alternative cell types in 3D to mimic tissue structures
- To investigate invasion and migration of different cell types amongst each other

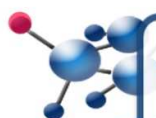
### 5 Assembly option 2D / 3D co-culture in multi-well plate



**Description**  
One 2D and one 3D culture layered in direct contact with one another

**Application**

- Study the direct interaction of cells in contact with one another
- To establish layers of alternative cell types in 3D to mimic tissue structures
- To investigate invasion and migration of different cell types amongst each other



UK & Rest of World  
184 Milton Park, Abingdon  
OX14 4SE, Oxon, UK  
Tel: +44 (0) 1235 828 200  
Fax: +44 (0) 1235 820 482

Switzerland  
Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
Tel: +41 (0) 91 604 55 22  
Fax: +41 (0) 91 605 17 85

Deutschland  
Bockenheimer Landstr. 17/19  
60325 Frankfurt/Main  
Tel: +49 (0) 69 779099  
Fax: +49 (0) 69 13376880

United States  
23591 El Toro Rd, Suite #167  
Lake Forest, CA 92630  
Tel: +1 800 987 0985  
Fax: +1 949 265 7703

amsbio  
info@amsbio.com

Guidelines for the preparation of co-culture using Alvetex 12 well plates (AMS.AVP002) and alvetex 15 mm well inserts (AMS.AVP005-34):

1. **Day 0:** Setup feeder layer cultures in the bottom of 12-well plates (Product code AMS.AVP002) (no well inserts present). Seed cells at required density and incubate with media until Day 2, changing media if/when required.
2. **Day1:** Take the desired number of well inserts (Product code AMS.AVP005-34 -15mm in diameter - with wing extensions removed) and hydrate them by dipping them into a beaker containing 70% ethanol. Place wells into a fresh 12-well plate. Add 3ml of media (per well) to the well inserts to wash them. Aspirate and repeat washing step.
3. Aspirate all media from the well inserts and the wells themselves. Seed the cells onto the centre of each disc in a small volume of media (e.g. 50-75ul containing  $0.25-0.5 \times 10^6$  cells, depending on cell type). Take care not to touch the disc with the pipette tip, to avoid tearing.
4. Place covered plates into a humidified incubator at 37% with 5% CO<sub>2</sub> for at least 15 minutes. This is to allow the cells to settle and adhere to the disc.
5. Fill up the wells carefully beside the insert, so the media comes up from the bottom to gently contact the alvetex disc and gradually floods the insert itself (3-4 ml).
6. Incubate overnight at 37% with 5% CO<sub>2</sub>.
7. **Day 2:** Take 12-well plates containing the feeder layers and remove media (reuse if required).
8. Take 12-well plates containing well inserts and remove media (reuse later if required).
9. Using sterile forceps place well inserts over the feeder layers.
10. Incubate with appropriate media (using the method described in step 5.) capable of sustaining both cell layers.
11. Maintain as frequently as required.