

# Example Protocols for the Co-Culture of either the SW620 or the SW480 Cell Line with the 3T3 Cell Line on Alvetex™ Scaffold in Well Insert Formats

## Introduction

Alvetex Scaffold is currently available in four different cell culture formats: 24 well plate (AVP006), Introduction

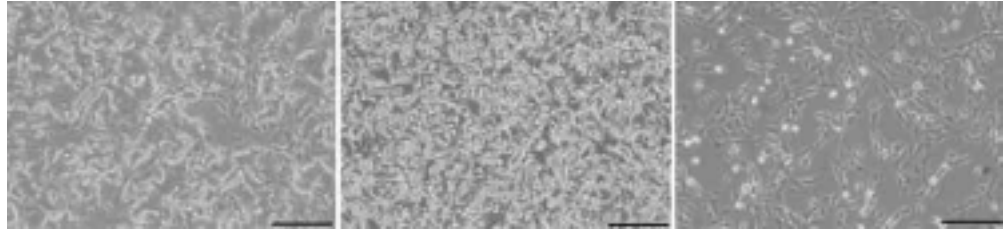
Alvetex Scaffold is currently available in four different cell culture formats: 24 well plate (AVP006), 12 well plate (AVP002), 6 well insert (AVP004), and 12 well insert (AVP005). 24 well and 12 well plates are suitable for shorter term cultures and for applications where limited cell penetration into the scaffold is required. Well insert formats generally support longer term cultures and deeper cell penetration into the scaffold. They also provide for conveniently tailored media set ups (see the Quick Start Protocol).

6 well inserts can be placed in conventional 6 well plates, while 12 well inserts can be placed in either 6 well plates or 12 well plates, depending on media requirements. Alternatively, both insert types can be housed in the dedicated Well Insert Holder in Deep Well Petri Dish (AVP015) to allow for increased media volumes and prolonged cell culture. The availability of two different Alvetex Scaffold sizes enables choice on the basis of desired culture size and cell expenditure.

## Methods

### Preparation for 3D Cell Culture on Alvetex Scaffold

1. SW480, SW620 and 3T3 cells were routinely maintained in T-75 flasks.



**Figure 1.** Phase contrast micrographs of SW480 cells (left), SW620 cells (middle) and 3T3 cells (right) grown in conventional 2D culture plates. Images show cells at high confluency. Scale bars: 200  $\mu$ m.

2. For SW480 cells and SW620 cells, complete growth media consisted of: DMEM High glucose supplemented with 10 % v/v heat inactivated FBS, 2  $\mu$ m L-glutamine and 100 U/mL Penicillin/Streptomycin.
3. For 3T3 cells, complete growth media consisted of: DMEM High glucose supplemented with 10 % v/v FBS, 2  $\mu$ m L-glutamine and 100 U/mL Penicillin/Streptomycin.
4. Cells were harvested by trypsinisation and centrifuged for 5 minutes (1000 rpm). The supernatant was discarded and the cell pellet was re-suspended in an appropriate volume of media for cell counting by Trypan Blue.
5. Cells were re-suspended at a concentration of  $1.0 \times 10^7$  cells/mL for seeding.

**Note:** 3T3 cells are seeded on Alvetex Scaffold 7 days prior to seeding SW480 cells (or SW620 cells).

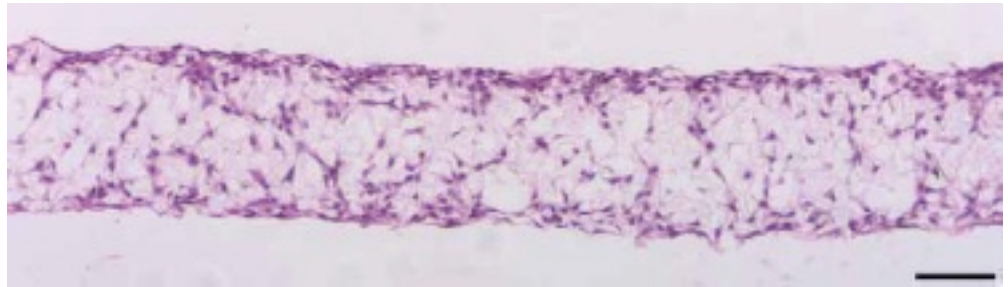
### 6 well Insert Format, AVP004

1. Alvetex Scaffold 6 well inserts in 6 well plate format were prepared for seeding by dipping in 70 % ethanol and washed twice with media (7 mL per well).
2. 50  $\mu$ L of the 3T3 cell suspension was added in the centre of the Alvetex Scaffold disc, which was equivalent to  $0.5 \times 10^6$  cells per well.
3. The plate was incubated for a minimum of 15 minutes at 37 °C with 5 % CO<sub>2</sub> to allow the cells to settle into the scaffold.
4. 3T3 media was added to each well to a total volume of 10 mL taking care not to dislodge cells from Alvetex Scaffold.

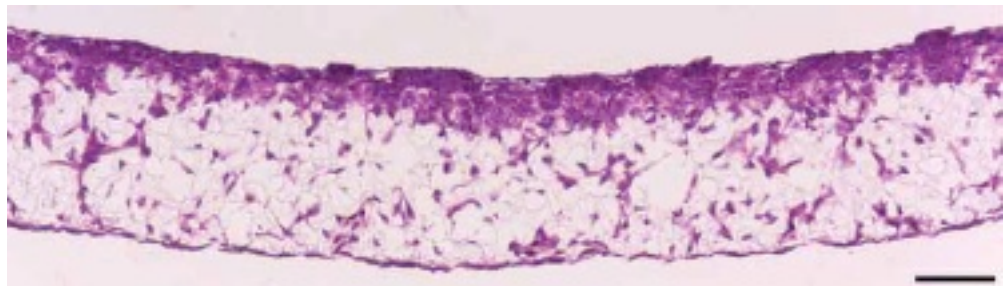
5. Plates were re-incubated and maintained for a further 7 days by complete 3T3 media exchange after every 2-3 days.
6. After 7 days, inserts were transferred to deep well Petri dish holders in deep well Petri dishes (AVP015) and dishes were filled from the outside of the insert with enough SW480/SW620 medium to allow the medium to rise inside the insert and cover the substrate, but not to goove the sides of the inserts, i.e. 50 mL  $\pm$  5 mL, as described for feeding above and below separately in the Quick Start protocol.
7. 100  $\mu$ L of either the SW480 cell suspension or the SW620 cell suspension was added in droplets over the surface of the Alvetex Scaffold disc, which was equivalent to  $1 \times 10^6$  cells per well.
8. The Petri dish was incubated overnight at 37 °C with 5 % CO<sub>2</sub> to allow the cells to settle on top of the scaffold.
9. The following morning SW480/SW620 media was added to each Petri dish to a total volume of 70 mL taking care not to dislodge cells from Alvetex Scaffold.
10. Petri dishes were re-incubated and maintained by complete SW480/SW620 media exchange after every 2-3 days.

**Note:** This method can be applied to the use of Alvetex Scaffold in 12 well insert format, AVP005. Adjust cell seeding and media volumes according to the guidelines provided in the Quick Start Protocol.

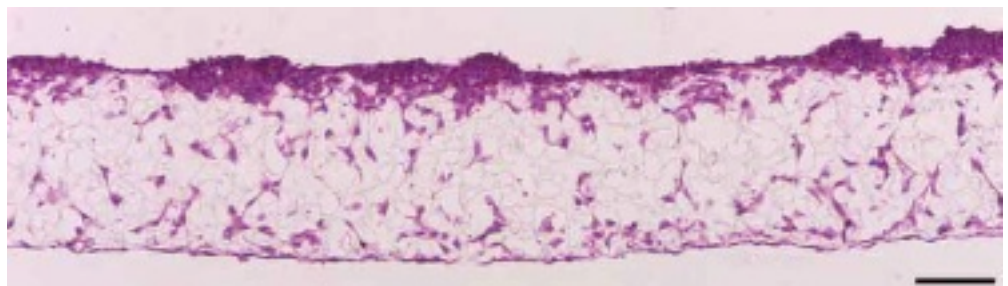
## Example Data: 6 well insert format, AVP004



**Figure 2.** Brightfield micrograph showing the structure of 3T3 cells cultured for 7 days on 22 mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10  $\mu\text{m}$ ) and counterstained with haematoxylin and eosin. Scale bar: 100  $\mu\text{m}$ .



**Figure 3.** Brightfield micrograph showing the structure of SW480 cells co-cultured for 7 days with established 3T3 cells on 22mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10  $\mu\text{m}$ ) and counterstained with haematoxylin and eosin. Scale bar: 100  $\mu\text{m}$ .



**Figure 4.** Brightfield micrograph showing the structure of SW620 cells co-cultured for 7 days with established 3T3 cells on 22 mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10  $\mu\text{m}$ ) and counterstained with haematoxylin and eosin. Scale bar: 100  $\mu\text{m}$ .

## Notes

---

### REPROCELL USA Inc

9000 Virginia Manor Road  
Suite 207  
Beltsville, MD 20705  
USA

Tel: +1 301 470 3362  
Email: [info-us@reprocell.com](mailto:info-us@reprocell.com)

### REPROCELL Europe Ltd

Thomson Pavilion, Todd Campus  
West of Scotland Science Park  
Acre Road  
Glasgow G20 0XA  
UK

Tel: +44 (0)141 465 3460  
Email: [info-emea@reprocell.com](mailto:info-emea@reprocell.com)

### REPROCELL India Ltd

3-1-135/1A, CNR Complex  
Mallapur  
Hyderabad 500 076  
Telangana  
India

Tel: +44 (0)141 465 3460  
Email: [Bhargavi.Gurram@reprocell.com](mailto:Bhargavi.Gurram@reprocell.com)

### REPROCELL Inc (Japan)

MetLife Shin-yokohama 381, Bldg. 9F  
3-8-11, Shin-yokohama  
Kohoku-ku, Yokohama  
Kanagawa 222-0033  
Japan

Tel: +81 45 475 3887  
Email: [info-asia@reprocell.com](mailto:info-asia@reprocell.com)