



## Example Protocols for the Culture of the CHO-K1 Cell Line on Alvetex® Scaffold in Well Plate and Well Insert Formats

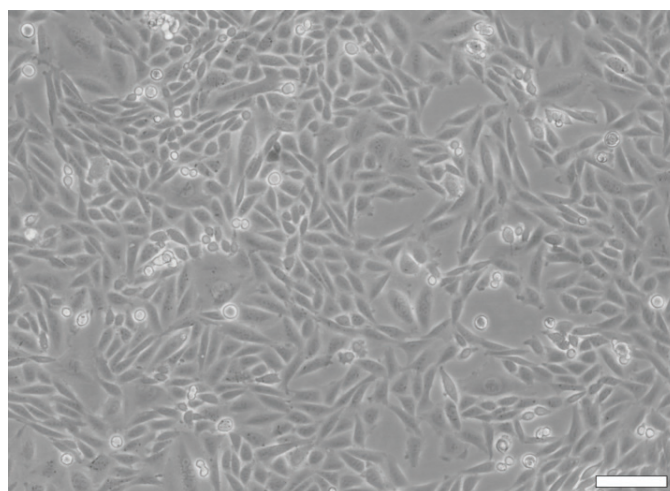
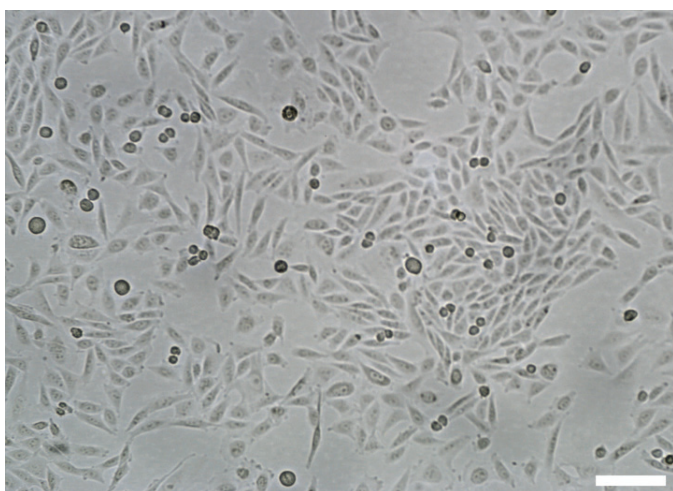
### Introduction

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 Quick Start Protocol, [www.reinnervate.com/alvetex/alvetex-documentation/protocols](http://www.reinnervate.com/alvetex/alvetex-documentation/protocols)). The availability of two different well insert formats enables choice on the basis of desired culture size and cell expenditure. 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.

### Methods

#### Preparation for 3D cell culture on Alvetex Scaffold

1. CHO-K1 cells were routinely maintained in T-75 flasks.



**FIGURE 1.** Phase contrast micrographs of CHO-K1 cells grown in conventional 2D culture flasks. Images show cells at low (left) and high (right) confluency. Scale bars: 100µm.

2. Complete medium consisted of: F-12K nutrient mixture (Kaighn's modification) supplemented with 10 % v/v FBS and 100 µg/ml Penicillin and 10 µg/ml Streptomycin.

**Note:** F12-K nutrient medium already contains L-glutamine.

3. Cells were harvested by trypsinisation and centrifuged for 5 minutes (1000rpm). The supernatant was discarded and the cell pellet was re-suspended in appropriate volume of media for cell counting by Trypan Blue.
4. Cells were re-suspended at a concentration of  $8 \times 10^6$  cells / ml for seeding.



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5. Follow one of the below methods according to your choice of Alvetex Scaffold format.

### 12-well Plate Format, AVP002:

1. Alvetex Scaffold 12-well discs were prepared for seeding with a 70 % ethanol wash (2 ml per well) and subsequent media washes (twice with 3 ml of media each).
2. 125 µl of the cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent to  $1 \times 10^6$  cells per well.
3. The plate was incubated 30 minutes at 37 °C with 5% CO<sub>2</sub> to allow the cells to settle into the scaffold.
4. 4 ml of medium was added to each well taking care not to dislodge cells from Alvetex Scaffold.
5. Plates were re-incubated and maintained by complete medium exchange after every 1-2 days.

**Note:** This method can be applied to the use of Alvetex Scaffold in 24-well plate format, AVP006. Adjust cell seeding and media volumes according to the guidelines provided in the Quick Start Protocol.

### 6-well Insert Format, AVP004:

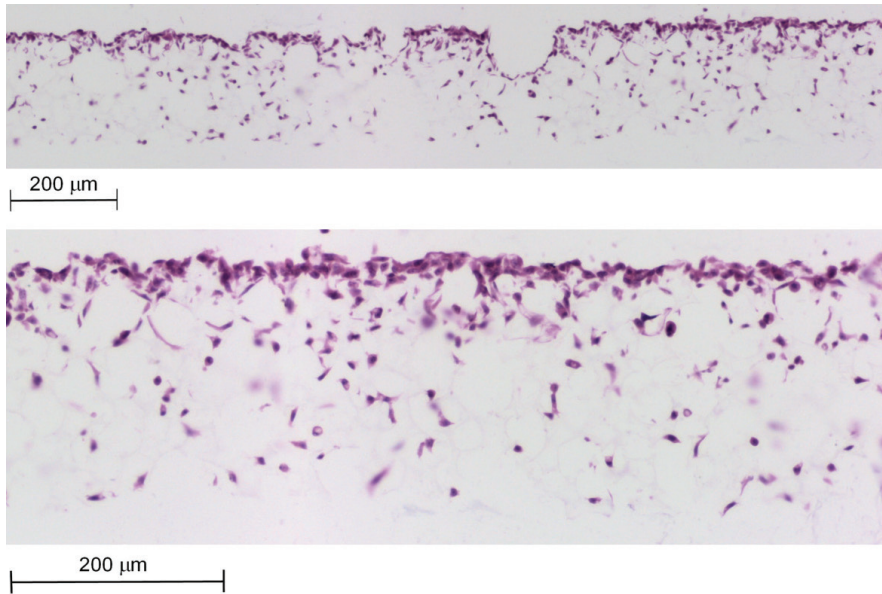
1. Alvetex Scaffold 6-well inserts in 6-well plate format were prepared for seeding by dipping in 70% ethanol and washed twice with media (7 ml per well).
2. 125 µl of the cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent to  $1 \times 10^6$  cells per well.
3. The plate was incubated 30 minutes at 37°C with 5% CO<sub>2</sub> to allow the cells to settle into the scaffold.
4. 10 ml of medium was added to each well taking care not to dislodge cells from Alvetex Scaffold.
5. Plates were re-incubated and maintained by complete medium 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.

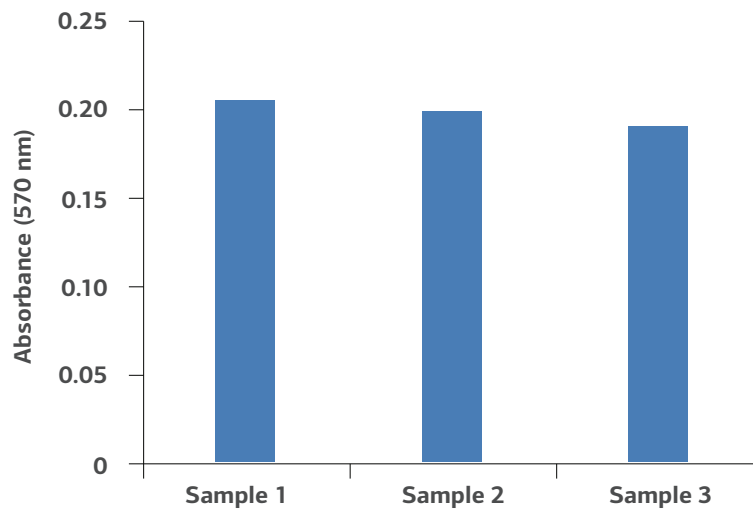
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## Example Data: 12-well plate format, AVP002



**FIGURE 2.** Brightfield micrographs showing the structure of CHO-K1 cells cultured for 3 days on 22 mm diameter Alvetex Scaffold discs presented in the 12-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.



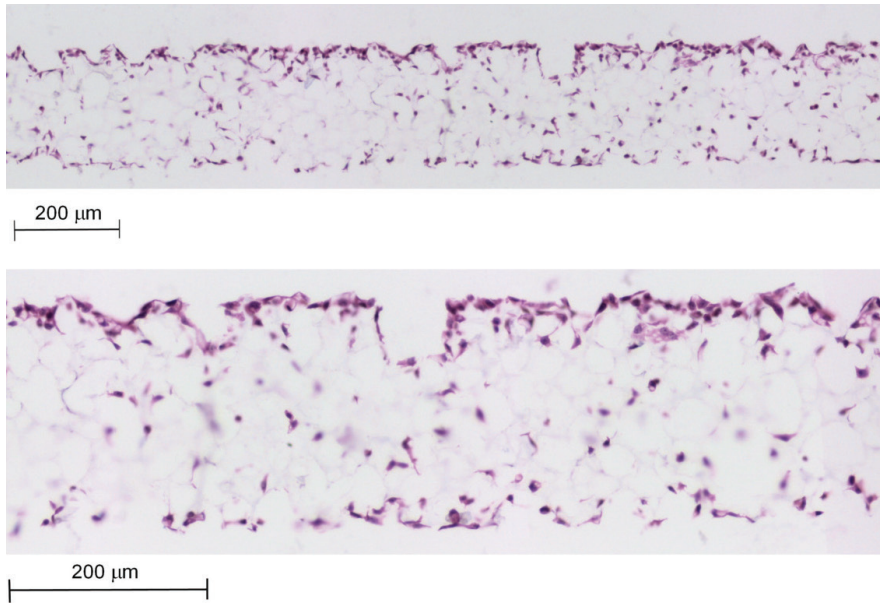
**FIGURE 3.** Biochemical analysis of cell viability using a standard MTT assay. Mean data from 3 sample replicates of CHO-K1 cells are shown. Cells were cultured for 3 days on 22 mm Alvetex Scaffold discs presented in the 12-well plate format.

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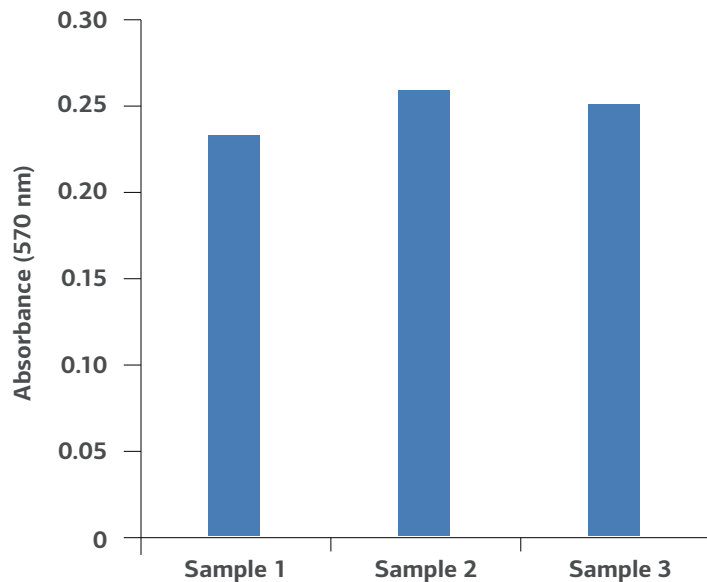
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## Example Data: 6-well insert format, AVP004



**FIGURE 4.** Brightfield micrographs showing the structure of CHO-K1 cells cultured for 4 days on 22 mm diameter Alvetex Scaffold discs presented in the 6-well insert in 6-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10µm) and counterstained with haematoxylin and eosin.



**FIGURE 5.** Biochemical analysis of cell viability using a standard MTT assay. Mean data from 3 sample replicates of CHO-K1 cells are shown. Cells were cultured for 3 days on 22 mm Alvetex Scaffold discs presented in the 12-well plate format.

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