Preparation for 3D cell culture on alvetex®:

1. HepG2 cells were routinely maintained in T-75 flasks.

2. Complete media consisted of: MEM media (Gibco 21090) supplemented with 10% v/v FBS, 2 mM L-glutamine and 100 U/ml Penicillin/Streptomycin.

3. 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.

4. Cells were re-suspended at a concentration of 1.6x10^7 cells/ml for seeding.

5. Alvetex® 6-well inserts were prepared for seeding by dipping in 70% ethanol and washed twice with 7 ml of media per well.

6. 125 µl of the cell suspension was added to the centre of the alvetex® disc, which was equivalent to 2x10^6 cells per well.

7. The plate was incubated 30 min at 37 °C with 5% CO₂ to allow the cells to settle into the scaffold.

8. 10 ml of media was added to each well taking care not to dislodge cells from alvetex®.

9. Plates were re-incubated and maintained by complete media exchange after every 2-3 days.
Example protocol for the culture of the HepG2 cell line on alvetex®
(22 mm disc in 6-well insert format, AMS.AVP004-32)

**Figure 2.** Brightfield micrographs showing the structure of HepG2 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in the 6-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. Data from 3 sample replicates of HepG2 cells are shown (n=3, mean ± SD). Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 6-well inserts in 6-well plate format.

AMSBIO is the global source for alvetex®.
alvetex® is a registered trade mark of and manufactured by Reinnervate.