

Protocol

Alvetex™ Scaffold 96 Well Plate: Quick Start

1. Description

REPROCELL's Alvetex Scaffold 96 well plate ([AVP009](#)) is comprised of a black plate, clear plastic base, with Alvetex Scaffold at the bottom of each well. The Alvetex Scaffold has been heat welded to the base of the wells in a process which does not alter its physical structure. Cells growing in 3D are exposed to culture medium from above only and predominantly reside in the top portion of the scaffold. As with 2D culture, the use of coating reagents to enhance cell growth can be considered (e.g. ECM proteins, poly-d-lysine).

Alvetex Scaffold 96 well plate technology is reproducible and compatible with a variety of *in vitro* cell viability assays and offers the advantage of assay multiplexing, which may not be possible when using alternative cancer cell biology models, such as soft agar clonogenic assays.

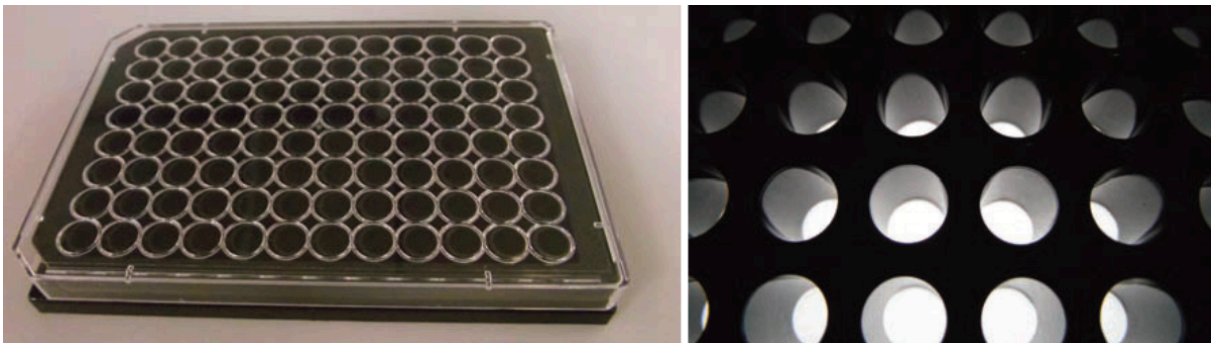


Figure 1. Alvetex Scaffold in 96 well plate format.

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2. Notes before starting

- Alvetex Scaffold 96 well plate is supplied gamma irradiated and sterile until opened.
- Prior to use, the sample wells must be rehydrated by the addition of 70 % ethanol (~100 μ L).
- The ethanol solution must then be removed by aspirating, sample wells washed once with PBS (200 μ L), and then once with the appropriate culture media (200 μ L), or alternatively 2 \times media washes (200 μ L). (The PBS and media washes must be carried out immediately after aspiration of the ethanol solution to avoid the scaffold drying out.)
- The plate can now be used immediately for seeding of cells or placed in the incubator (with wells containing culture media), until the cells are ready for seeding.

3. Seeding Cells on Alvetex Scaffold 96 well plates

For the best results it is recommended to optimise cell seeding density in the first instance. We have previously demonstrated that between 5,000 and 100,000 cells seeded per well in the final culture volume (e.g. 200 μ L per well). This will depend on the nature of the cell type and its appropriate growth conditions. Typically seeding cells on the range of 10-30,000 cells per well is a good starting point. Note that a 1 hour incubation at room temperature after seeding but prior to culture at 37 $^{\circ}$ C may improve the uniformity of cell coverage on each scaffold. Media changes should ideally be performed every \geq 2 days to reduce any temperature variability which may be caused by repeated removal of the plate from the incubator.

Note: Temperature variations, edge effects and stacking: As with conventional 2D culture in the 96 well format, plates either cultured in or removed from an incubator at 37 $^{\circ}$ C in tall stacks, will take longer to equilibrate to the surrounding temperature than plates arranged separately. In addition, it is well known that inefficient temperature equilibration can result in temperature gradient effects between wells in the centre and wells at the edge of a plate. Plate stacking may exacerbate such cross-plate temperature gradients, as well as causing differential gradient patterns between plates in the same stack. This may affect assay performance and cell migration toward the edge of the wells when seeding. This is a generic feature that can occur in standard microtitre plates and is not due to the presence of Alvetex Scaffold.

4. Example data derived from Alvetex Scaffold 96 well plates

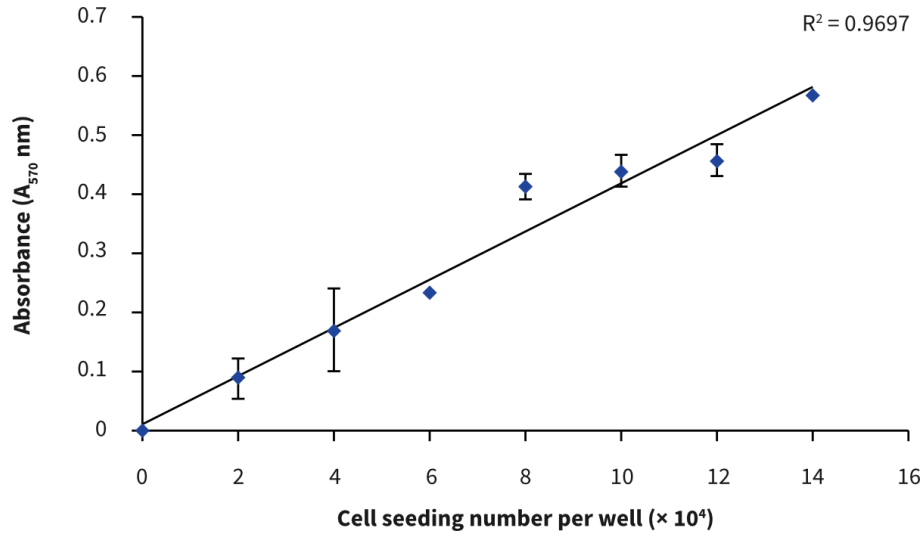


Figure 2. Cell number standard curve for the HepG2 cell line using the MTT cell viability assay. HepG2 cells were seeded at a range of densities on to Alvetex Scaffold 96 well plate format and cultured for 24 hours prior to analysis; linear increase in absorbance with increasing cell density was observed for up to 140,000 cells per well. Data from 3 sample replicates are shown ($n = 3$, mean \pm SD).

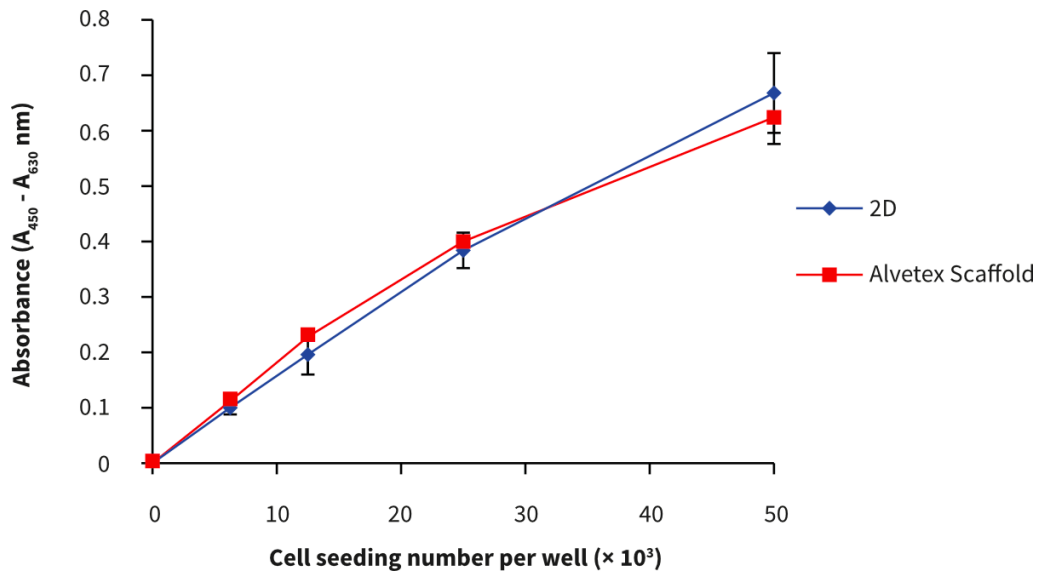


Figure 3. Cell number standard curve for the HepG2 cell line using the XTT cell viability assay. HepG2 cells were seeded at a range of densities on to Alvetex Scaffold 96 well plate format and cultured for 24 hours prior to analysis. Triplicate cultures were prepared at a range of seeding densities ($n = 3$, mean \pm SD). Specific absorbance values were comparable between 2D controls and 3D Alvetex Scaffold cultures up to a seeding density of 50,000 viable cells per well.