



Media Volumes Guide for Different Feeding Options in Alvetex® Scaffold in Well Insert formats

Alvetex Scaffold Insert formats:

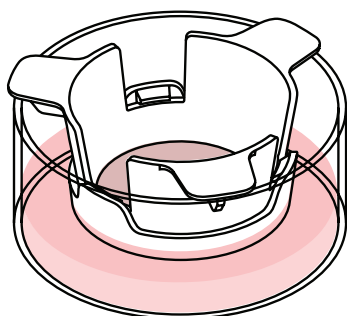
Alvetex Scaffold inserts are currently available in the following formats; 12-well inserts (AVP005) and 6-well inserts (AVP004), suitable for longer-term and co-culture experiments and air/liquid interface set-ups. Inserts are compatible with standard tissue culture plates and with Reinnervate's well insert holder in a deep Petri dish (AVP015), designed for demanding cell types to provide increased volumes of culture media. 12-well inserts fit into both 6- and 12-well plates (to fit into 12-well plates the extended arms must be snapped off). When deciding which Alvetex Scaffold format to use, the following factors should be considered in combination:

- Cell type and duration of experiment,
- The desired depth of cell penetration into the 3D scaffold,
- The type of assay or end point analysis to be performed.

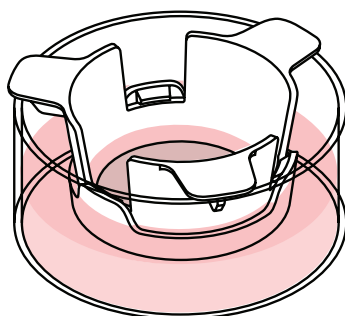
For Advice on Alvetex handling prior to use, cell seeding densities and monitoring of cell attachment for all available inserts and plate formats, please refer to our Quick Start Protocol at <http://www.reinnervate.com/alvetex/alvetex-documentation/protocols>

Media Volume Guide :

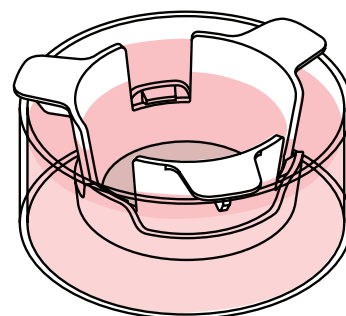
Alvetex inserts have been designed to allow for three media feeding options, each suited to different 3D culture applications as described in Figure 1. A guide to the volume of media required both inside and outside the insert is presented in Table 1 for each feeding option and both 6-well inserts (AVP004) and 12-well insert (AVP005) formats.



i. Media from below only:
for cells grown in 3D at air-liquid interface.



ii. Media from above and below:
for routine 3D growth of cells with lower-average metabolic activity/ proliferation rate OR for experiments where cells are incubated with test substrate in top chamber only for permeability investigations.



iii. Media interconnected:
for routine 3D growth of cells with high metabolic activity/ proliferation rate

Figure 1: Media filling levels and well insert configurations



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Using Alvetex Scaffold well inserts	Media volumes for different feeding options					
	Below only		Above and below separately		Above and below interconnected	
	Insert	Well	Insert	Well	Insert	Well
6-well insert in 6-well plate	None	2.4 ml	Up to 1.1 ml	2.4 ml to 4.2 ml	1.1ml to 4 ml	4.2 ml to 7 ml
12-well insert in 6-well plate	None	3.3 ml	Up to 0.5 ml	3.6 ml to 6.4 ml	0.5 ml to 1.9 ml	6.4 ml to 10.7 ml
12-well insert in 12-well plate	None	1.2 ml	Up to 0.5 ml	1.2 ml to 1.4 ml	0.5 ml to 1.9 ml	1.4 ml to 2.3 ml

Table 1: Media volumes for different Alvetex Scaffold feeding options. For the 'Below only' option, the insert should just be in contact with the media below. Volumes are approximate and will need to be adjusted by the end user.

For research use only. Not for use in diagnostic procedures.



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