

Protocol

Media Volumes Guide for Different Feeding Options in Alvetex™ Scaffold in Well Insert Formats

1. Introduction

Alvetex Scaffold inserts are currently available in the following formats; 24 well inserts ([AVP012](#)), 12 well inserts ([AVP005](#)), and 6 well inserts ([AVP004](#)). Alvetex well inserts are suitable for longer-term and co-culture experiments and air/liquid interface set-ups. Inserts are compatible with standard tissue culture plates and with REPROCELL's well insert holder in a deep Petri dish ([AVP015](#)), designed for demanding cell types to provide increased volumes of culture media. Alvetex 6 well and 12 well formats can also be used in the Alvetex Perfusion Plate ([AVP011](#)).

Alvetex 24 well inserts and 12 well inserts are designed to fit two sizes of plate: the named well plate size and the next size up. When supplied, these inserts have “extender wings” attached to enable them to be supported on the wider rim of the next sized plates – snap the extender wings off, to fit the well inserts to into wells of their named plate size (see **Figure 1**).

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 [Alvetex Scaffold Quick Start Protocol](#).

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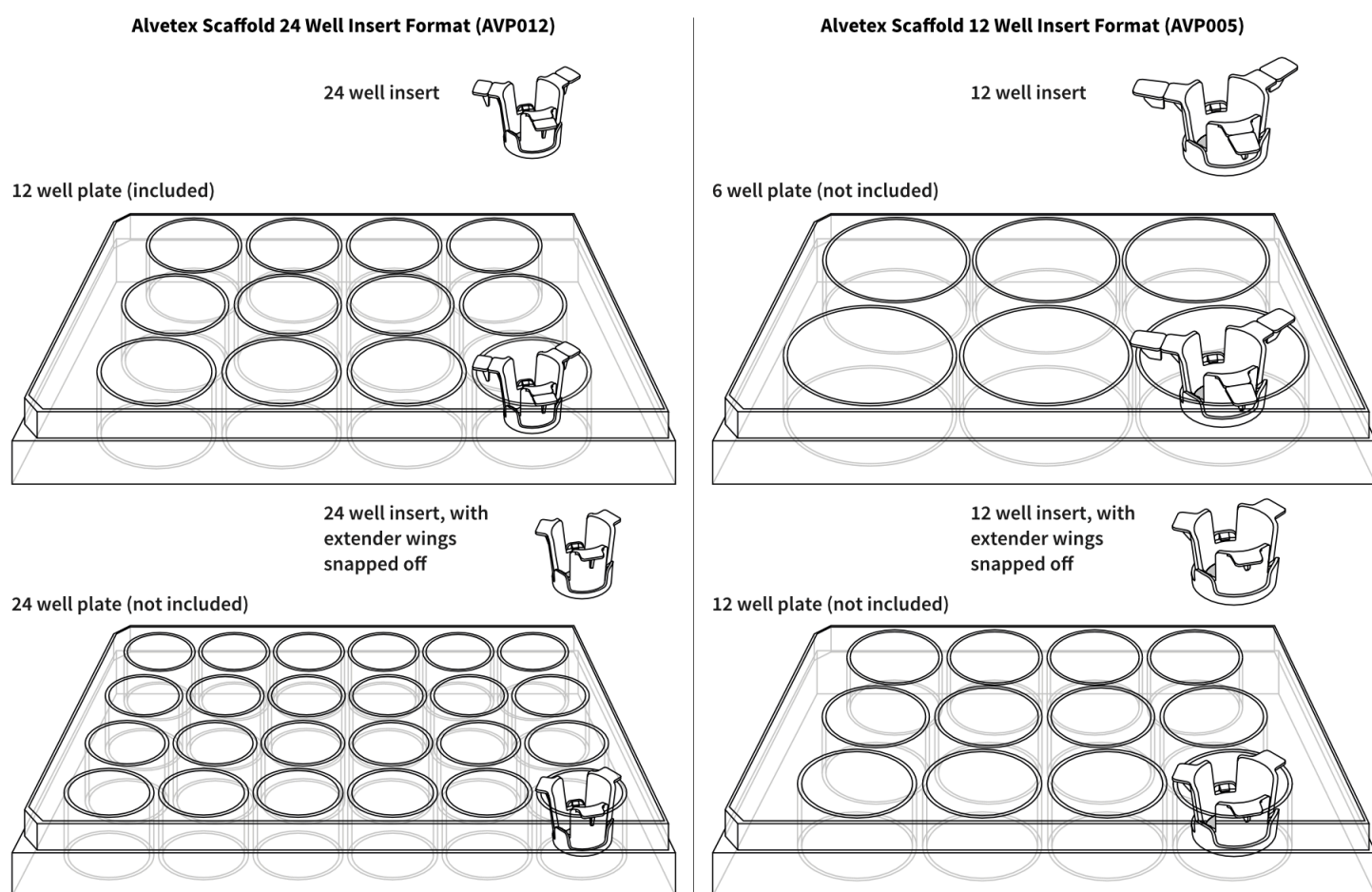
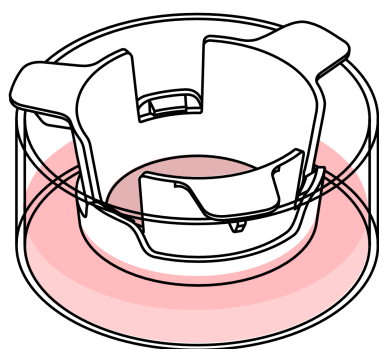


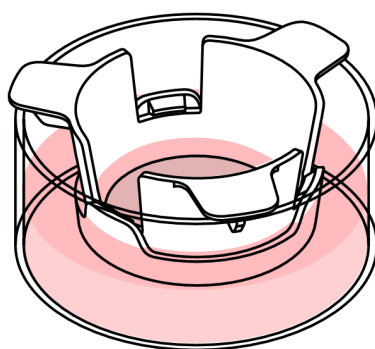
Figure 1. Fitting of Alvetex Scaffold 24 well inserts (AVP012) and 12 well inserts (AVP005) in multiwell plates.

2. Media volumes guide

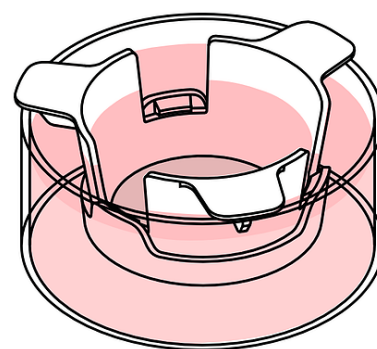
Alvetex inserts have been designed to allow for three media feeding options, each suited to different 3D culture applications as described in **Figure 2**. A guide to the volume of media required both inside and outside the insert is presented in **Table 1** for each feeding option and both 6 well inserts (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 2. Media filling levels and well insert configurations for the 6 well insert (AVP004) in a 6 well plate. (Other formats can be filled similarly.)

Using Alvetex Scaffold well inserts	Media volumes for different feeding options					
	(i) Media from below only		(ii) Media from above and below		(iii) Media interconnected	
	Insert	Well	Insert	Well	Insert	Well
24 well insert (AVP012) in 24 well plate	None	0.8 mL to 1 mL	< 0.4 mL	0.8 mL to 1 mL	> 0.4 mL	1.8 mL to 2.3 mL
24 well insert (AVP012) in 12 well plate	None	1.5 mL to 2.5 mL	< 0.4 mL	1.5 mL to 2.5 mL	> 0.4 mL	3.5 mL to 4.5 mL
12 well insert (AVP005) in 12 well plate	None	1.5 mL to 1.8 mL	< 0.5 mL	1.5 mL to 1.8 mL	> 0.5 mL	3.5 mL to 4.5 mL
12 well insert (AVP005) in 6 well plate	None	3.6 mL to 5.5 mL	< 0.5 mL	3.6 mL to 5.5 mL	> 0.5 mL	8.5 mL to 11 mL
6 well insert (AVP004) in 6 well plate	None	3.6 mL to 5 mL	< 1.1 mL	3.6 mL to 5 mL	> 1.1mL	8.5 mL to 11 mL

Table 1. Media volumes for different Alvetex Scaffold feeding options. For **(i.)** Media from below only, the insert should just be in contact with the media below. Volumes are approximate and will need to be adjusted by the end user.