

Protocol

Media Volumes Guide for Different Feeding Options in the Alvetex® Advanced Tissue Bioengineering System

1. Introduction

Alvetex Advanced inserts (containing Alvetex Scaffold membrane) are currently available in the 15 mm diameter membrane disc format (AVP022). Alvetex Advanced inserts are suitable for longer-term 3D cell culture, co-culture, tissue model bioengineering experiments, and air/liquid interface set-ups.

Besides being compatible with various standard, commercially available 6 well tissue culture plates, the **Alvetex Advanced Tissue Bioengineering System** has a range of plates with larger wells for greater media volumes, designed for longer term culture and the maintenance of tissue models. When supplied with the required height handles and plates, Alvetex Advances inserts can be used in multiple combinations allowing for long-term culture, transport, and compound addition (see **Figures 1-5**).

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 Alvetex plasticware formats, please refer to our [Alvetex Scaffold Quick Start Protocol](#) as a starting point.

Table 1: The Alvetex Advanced Tissue Bioengineering System.

Product Name	Product Number	Pack Sizes
Alvetex Advanced 15mm Modular Inserts containing Alvetex Scaffold Membrane In blister packed units of 6 inserts in an insert holder, with lid	AVP022	6, 12, 24, 48, 60, or 96 inserts
Low handles for Alvetex Advanced modular inserts	AVP023	12, 24, 48, 60, or 96 low handles
Medium handles for Alvetex Advanced modular inserts	AVP024	12, 24, 48, 60, or 96 low handles
High handles for Alvetex Advanced modular inserts	AVP025	12, 24, 48, 60, or 96 low handles
Advanced deep plates 6 well, with lid	AVP026	5, 10, or 15 plates
Advanced single well plates 50 ml, with lid	AVP027	5, 10, or 15 plates
Advanced single well plates 75 ml, with lid	AVP028	5, 10, or 15 plates
Advanced single well plates 125 ml, with lid	AVP029	5, 10, or 15 plates
6-well handle holder for use with Advanced single well plates	AVP030	2, 4, or 10 holders
6-well holder for Alvetex Advanced modular inserts, with lid	AVP031	1, 2, or 10 holders

Note: All products are sterile gamma irradiated.



Figure 1. Alvetex Advanced 15 mm insert containing Alvetex Scaffold membrane. Sterile blister packed units contain 6 inserts, 1 insert holder, and 1 lid. (Product number AVP022.)

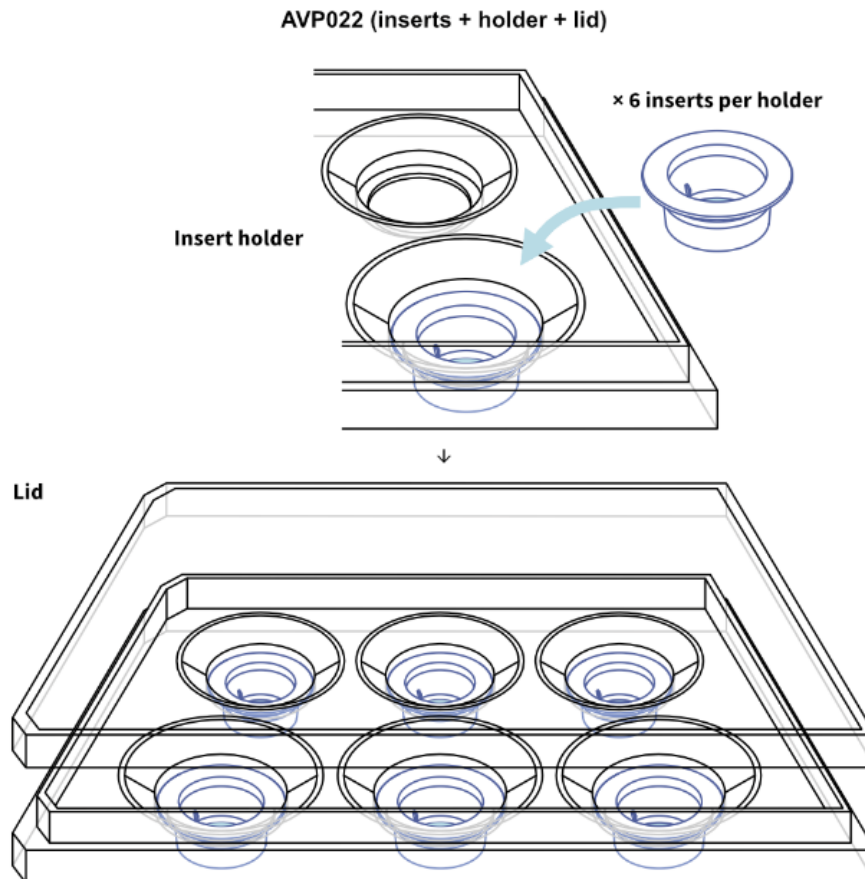
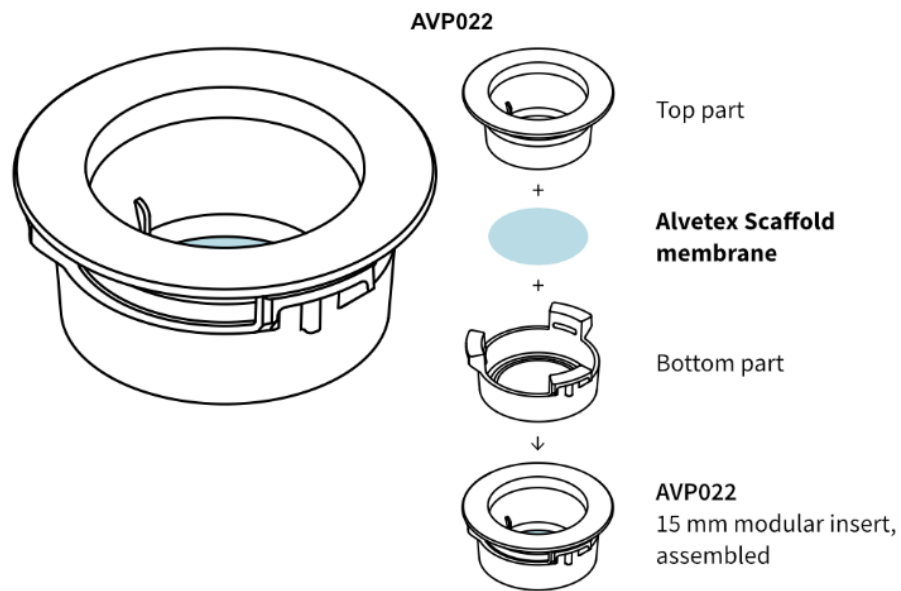


Figure 2. Diagram of Alvetex Advanced 15 mm insert containing Alvetex Scaffold membrane. Sterile blister packed units contain 6 inserts, insert holder, and lid. (Product number AVP022.)

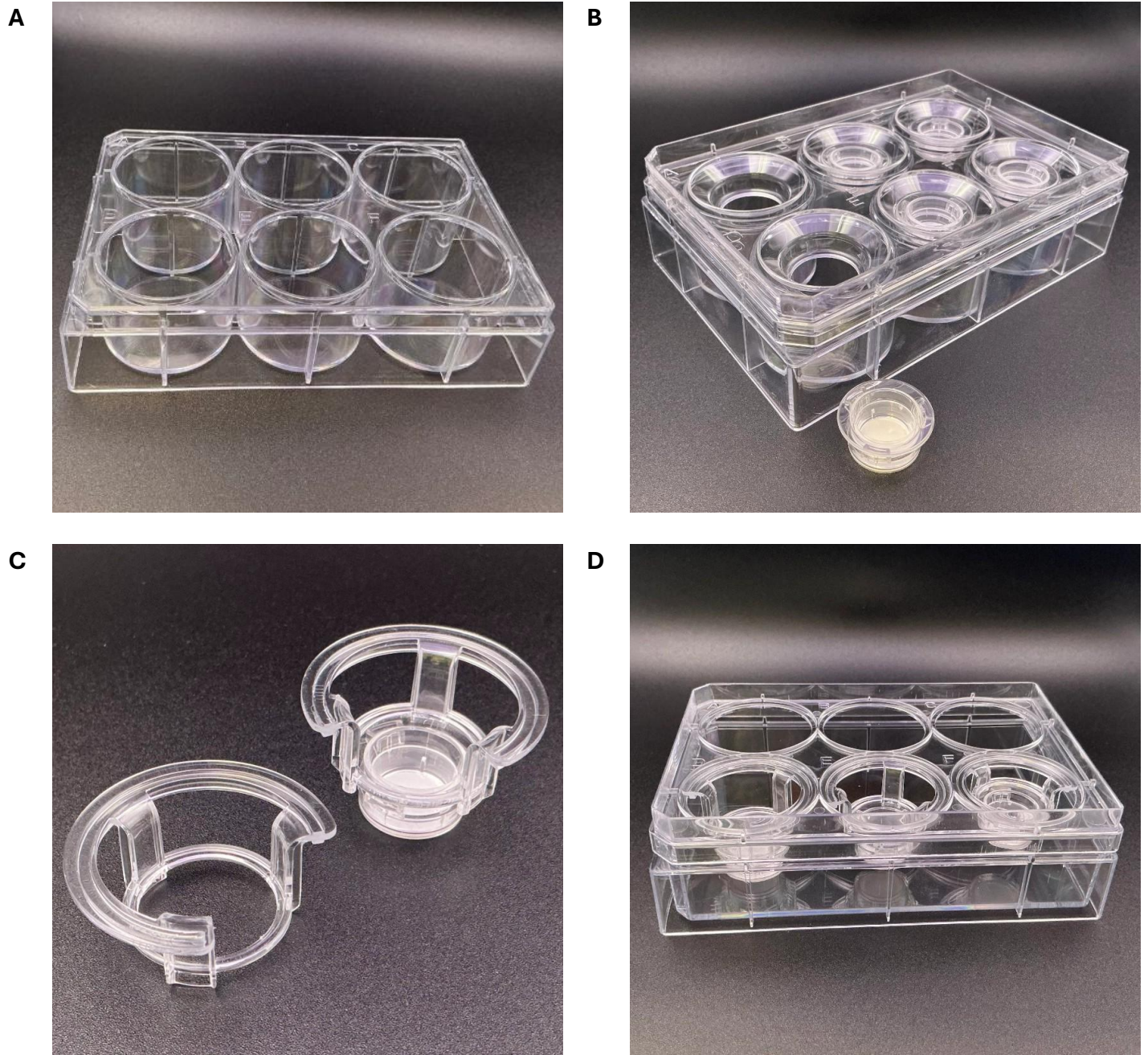


Figure 3. Example products in the Alvetex Advanced Tissue Bioengineering System. **(A.)** Advanced deep 6 well plate (AVP026). **(B.)** Alvetex Advanced 15 mm inserts in insert holder (AVP022), in Advanced deep 6 well plate (AVP026). **(C.)** Medium handle for Alvetex Advanced inserts (AVP024). **(D.)** Low (AVP023), medium (AVP024), and high (AVP025) handles in handle holder (AVP030) within Advanced single well plate, 125 ml (AVP029).

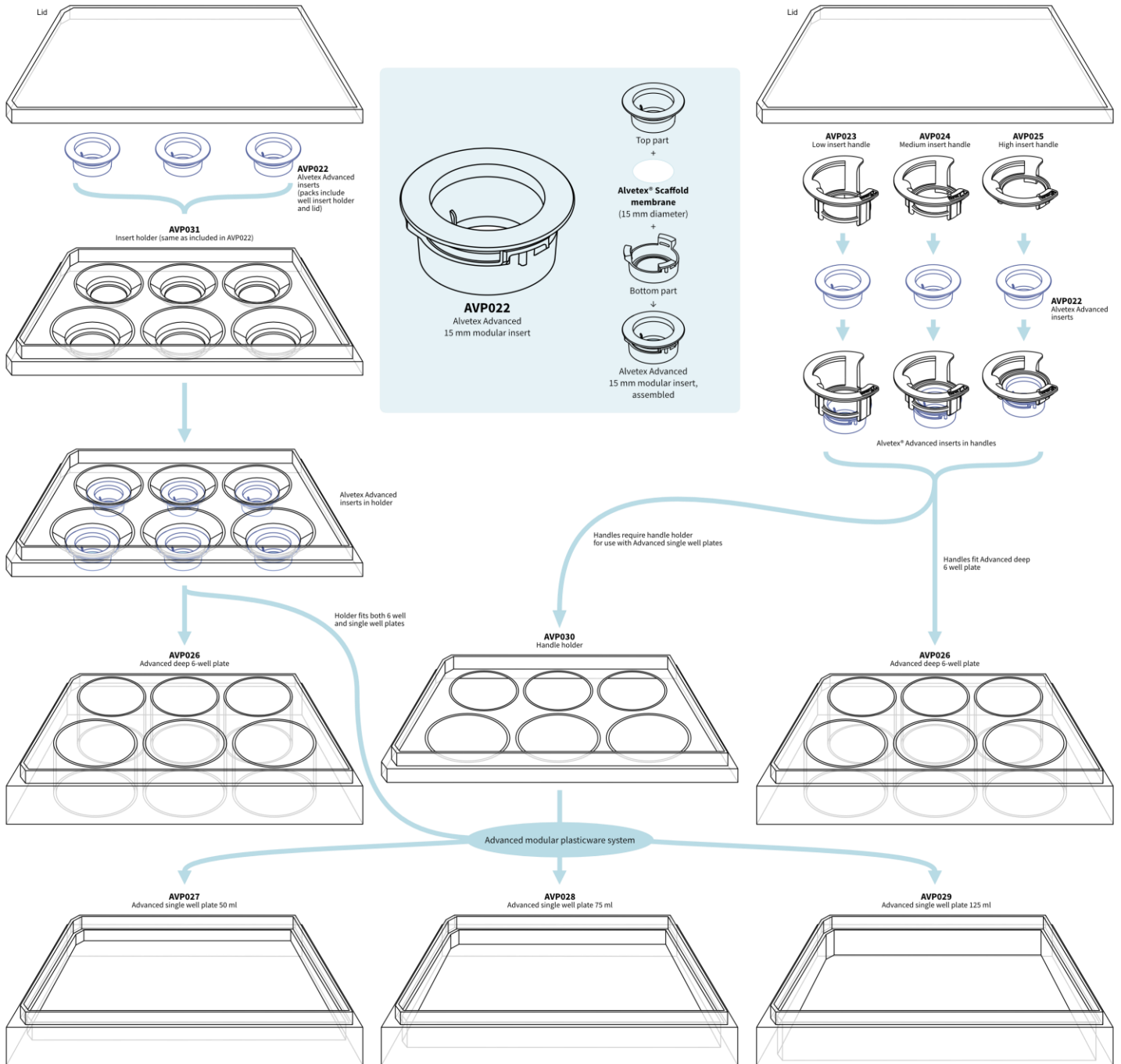


Figure 4. Overview diagram of the Alvetex Advanced Tissue Bioengineering System.

2. Media volumes guide

2.1 Feeding cells from below only (for air-liquid interface set-ups)

The Alvetex Advanced insert holder (that comes with the inserts in AVP022 and is also available separately as AVP031) is designed for bioengineered skin models where culture media feeds the cells from below only – the tissue model forming at the air-liquid interface. The insert holder tightly grips the Alvetex Advanced inserts around their circumference, creating a barrier between the top of the inserts and the bottom. This barrier will protect the media below from contamination by anything that is applied to the top surface of the bioengineered tissue model within the insert.

Table 2. Media volumes for different Alvetex Advanced insert (AVP022) feeding options using the **insert holder** (included with inserts in AVP022). The insert should just be in contact with the media below it. Volumes are approximate and will need to be adjusted by the end user.

Tissue culture plates	AVP022 Insert holder [1] + insert(s)	AVP023 Low handle + insert	AVP024 Medium handle + insert	AVP025 High handle + insert
Commercial 6 well plate (various)	5 - 6 ml	Incompatible [2]	Incompatible	3 - 4 ml
Advanced deep 6 well plate (AVP026)	15 - 20 ml	4 - 5 ml	6 - 7 ml	10 - 12 ml
Advanced single well plate, 50 ml (AVP027) [3]	53 - 57 ml	Incompatible	Incompatible	43 - 47 ml
Advanced single well plate, 75 ml (AVP028) [3]	93 - 97 ml	Incompatible	Incompatible	73 - 77 ml
Advanced single well plate, 125 ml (AVP029) [3]	133 - 137 ml	38 - 42 ml	68 - 72 ml	98 - 102 ml

Notes:

[1] The insert holder is included with inserts in AVP022. It is also available separately as AVP031.

[2] Incompatible because the [handle + insert] or [handle-holder + handle + insert] is too tall for the plate.

[3] Handles can fit directly onto the Advanced deep well plate (AVP026). But the handle holder AVP030 is required for the deep single well plates AVP027, AVP028 and AVP029.

Advanced deep 6 well plates (AVP026) present each insert with a separate culture medium well. Whereas the Advanced single well plates (50 ml, AVP027; 75 mL, AVP028; 125 mL, AVP029) each have one large rectangular well, where up to 6 inserts can share the same medium.

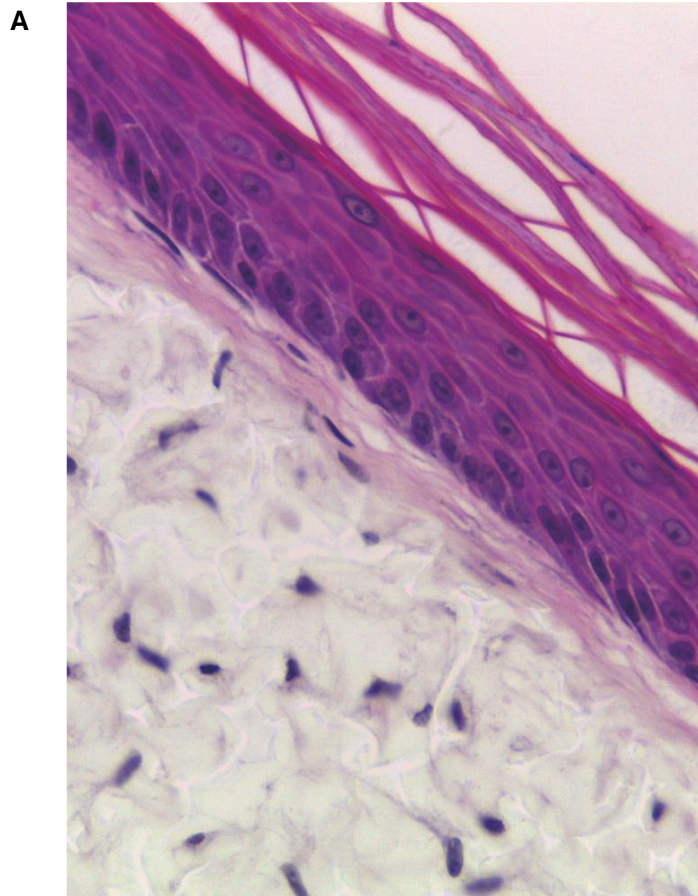
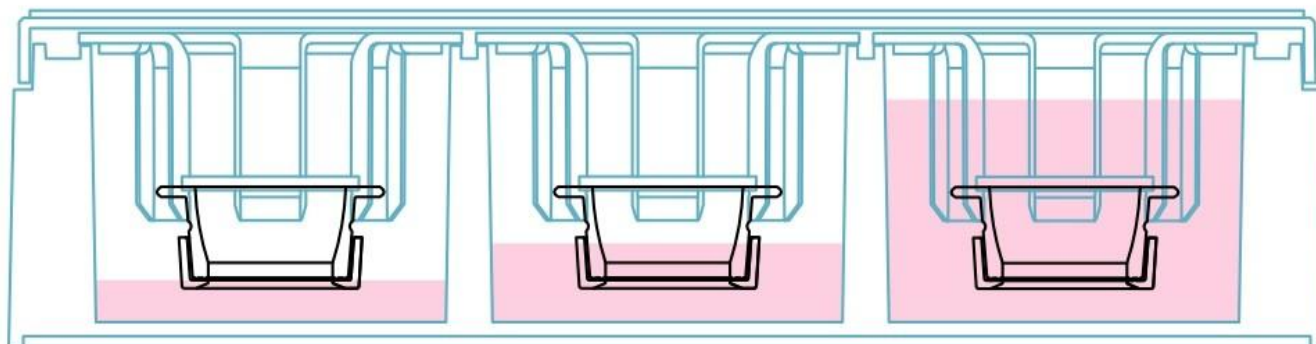


Figure 5: (A.) Bioengineered full thickness human skin model (sample prepared for histology and stained with H&E). (B.) Compatibility with instrumentation used in the clinic (example shows measurement of transepidermal water loss (TEWL)).

2.2. Feeding cells from below and above

Alvetex Advanced inserts in handles (AVP023, AVP024, or AVP025) have been designed to allow for three media feeding options, each suited to different 3D culture applications as exemplified in **Figure 6**. A guide to the volume of media required various configurations is presented in **Table 3** for each feeding option.



(i.) Media from below only

For cells in 3D at air-liquid interface.

(ii.) Media from above and below

For routine 3D growth of cells with lower-average metabolic/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/proliferation rate.

Figure 6. Media filling levels and well Alvetex Advanced insert + handle configurations with cradle handles only in 6-well plates or trays. Diagram shows insert (AVP022) in low handle (AVP023) as suspended in the Advanced deep 6 well plate (AVP026).

Table 3. Media volumes for different Alvetex Advanced insert (AVP022) feeding options using insert holder (AVP022; AVP031) or handles (low (25 mm), AVP023; medium (22 mm), AVP024; high (16 mm), AVP-25). For **(i.) Media from below only**, the insert should just be in contact with the media below it. Volumes are approximate and will need to be adjusted by the end user.

	Media volumes for different feeding options					
	(i) Media from below only		(ii) Media from above and below		(iii) Media interconnected	
	In insert	In well	In insert	In well	In insert	In well
Alvetex Advanced insert (AVP022) with low handle (AVP023)						
Advanced deep 6 well plate (AVP026)	None	4 - 5 mL	<1.5 mL	5 - 7 mL	>2 mL	18 - 20 mL
Advanced single well plate, 125 ml (AVP029)	None	38 - 42 mL	<1.5 mL	60 - 70 mL	>2 mL	130 - 140 mL
Advanced single well plate, 75 ml (AVP028)	N/A	N/A	<1.5 mL	43 - 47 mL	>2 mL	103 - 107 mL
Alvetex Advanced insert (AVP022) with medium handle (AVP024)						
Advanced deep 6 well plate (AVP026)	None	6 - 7 mL	<1.5 mL	8 - 12 mL	>2 mL	18 - 20 mL
Advanced single well plate, 125 ml (AVP029)	None	68 - 72 mL	<1.5 mL	93 - 97 mL	>2 mL	123 - 127 mL
Advanced single well plate, 75 ml (AVP028)	N/A	N/A	<1.5 mL	53 - 57 mL	>2 mL	N/A
Alvetex Advanced insert (AVP022) with high handle (AVP025)						
commercial 6 well plate (various)	None	3 - 4 mL	<1.5 mL	6 - 7 mL	>2 mL	10 - 12 mL
Advanced deep 6 well plate (AVP026)	None	10 - 12 mL	<1.5 mL	14 - 16 mL	>2 mL	18 - 20 mL
Advanced single well plate, 125 ml (AVP029)	None	98 - 102 mL	<1.5 mL	123 - 127 mL	N/A	N/A
Advanced single well plate, 75 ml (AVP028)	None	73 - 77 mL	<1.5 mL	103 - 107 mL	N/A	N/A
Advanced single well plate, 50 ml (AVP027)	None	43 - 47 mL	< 1.5 mL	73- 77 mL	N/A	N/A
Alvetex Advanced insert with insert holder (AVP022)						
commercial 6 well plate (various)	None	5 - 6 mL	<1.5 mL	7 - 8 mL	N/A	N/A
Advanced deep 6 well plate (AVP026)	None	15 - 20 mL	<1.5 mL	17 - 18 mL	N/A	N/A
Advanced single well plate, 125 ml (AVP029)	None	133 - 137 mL	<1.5 mL	151 - 154 mL	N/A	N/A
Advanced single well plate, 75 ml (AVP028)	None	93 - 97 mL	<1.5 mL	121 - 124 mL	N/A	N/A
Advanced single well plate, 50 ml (AVP027)	None	53 - 57 mL	< 1.5 mL	83 - 87 mL	N/A	N/A