Kit #2 – Happy Cell® ASM General Purpose Scale Up Kit

Culturing Cells in Happy Cell® ASM using 50 mL Bioreactors

User Guide

About Kit # 2 Happy Cell® ASM General Purpose Scale Up Kit

This kit is a simple and convenient means of growing 3D cells and micro-tissues to a larger scale.

It is suited to:

- the production of cells and/or 3D structures for applications such as proteomic and genomic analysis, as well as cell expansion studies
- supporting work conducted in and around the area of in vitro cell based assays, such as those seen in High Throughput and High Content Screening campaigns.

Catalogue Number: VHCK2

Components

- 1 x 25 mL bottle of Happy Cell® ASM supplied as a 4X concentrate
- 1 x 2 mL vial of Inactivation Solution (5 mg/mL)
- 5 x VHP low attachment 96 well microtiter plates with lids
- 2 x 50 mL sterile bioreactor tubes (with vented caps)

Additional Items Required

- Cell culture medium
- Desired cell culture additives e.g. Foetal Bovine Serum (FBS)
- A 37°C cell culture incubator

Please note Happy Cell® ASM contains Penicillin 10,000 I.U./mL and Streptomycin 10,000 µg/mL. We recommend any media used for diluting Happy Cell® ASM contains antibiotics at the same concentration.

We recommend reading our Happy Cell® ASM Preparation, optimization and Use Guide before commencing experimentation with this kit.

Storage and Expiry

Stable until expiry date on bottle if stored at 2-8°C. DO NOT FREEZE.

Ship at ambient temperature.

Preparation and Use

The Happy Cell® ASM Kit #2 can be used in two stages:

Stage 1

3D cell culture. Involves the use of one or both 50 mL bioreactor tubes.

- Wash and re-suspended cells in the desired density in Happy Cell® ASM. The recommended use for long term culture is 15 mL Happy Cell® media.
- Transfer cells to 50 mL bioreactor tube(s).
- Incubate at 37°C, gently agitating the cell suspensions every 24 hours
- Monitor the progress of your culture by gently mixing the suspension culture and removing 50 µL aliquots to a low attachment 96 well microplate.
- Using a microscope, cell number and 3D multi-cellular structure size can be observed.

Maintenance of cell cultures

- Happy Cell® ASM has been designed to sustain cells in long-term culture. Feed cells regularly to ensure healthy growth kinetics are maintained.
- Monitor the colour of the of the phenol red pH indicator in the media.
- Media should be replenished when a colour change from dark red to light red/yellow is observed.

Feeding Long term cultures with Happy Cell® ASM

Happy Cell® ASM cultures can be routinely fed with the same working concentration of Happy Cell® ASM by either one of two methods:

I. Topping up the level of Happy Cell® ASM in the cell culture vessel. We recommend adding 20% of the total volume of the culture (for example for a 100µl culture volume add 20µl fresh Happy Cell® ASM).

II. When necessary to maintain the same volume of liquid, or if there is insufficient room in the vessel for additional liquid, a small volume of Happy Cell® ASM (20% total volume) can be removed and replaced from the surface of the liquid. If you choose this option, ensure that you have not disturbed the cells prior to removing liquid (Figure 1).

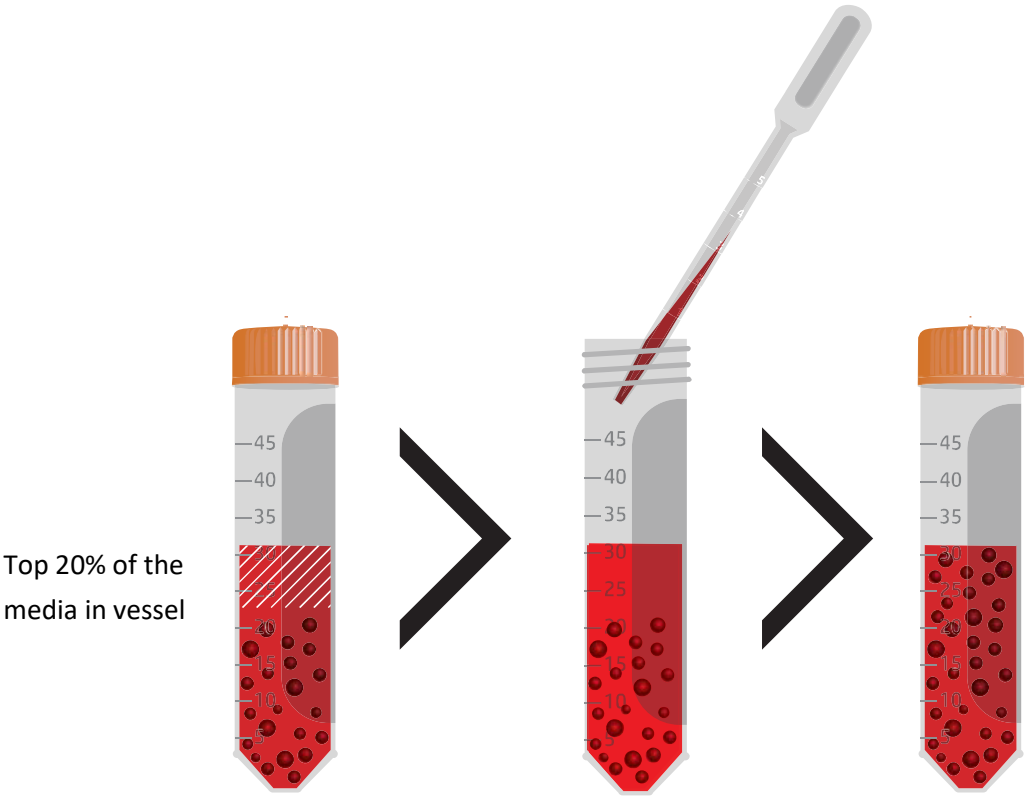

Figure 1. Media replacement using Happy Cell® ASM.

Step 1

When cellular aggregates or cells are grown in Happy Cell® ASM, they tend to occupy the lower 80% of the column of liquid in the culture vessel, leaving the 20% uppermost portion of the media free of any significant number

Step 2

Gently remove the uppermost 20% media and replace with fresh pre-warmed Happy Cell® ASM.

Step 3

When media replenishment has been completed, replace lid of the culture vessel and gently agitate until contents are fully mixed.

Stage 2

Harvesting and processing of 3D cultures.

Offers two alternatives. Cellular material may be:

- Harvested for analysis or processing;
- Re-plated for further experimentation **(Figure 2)**

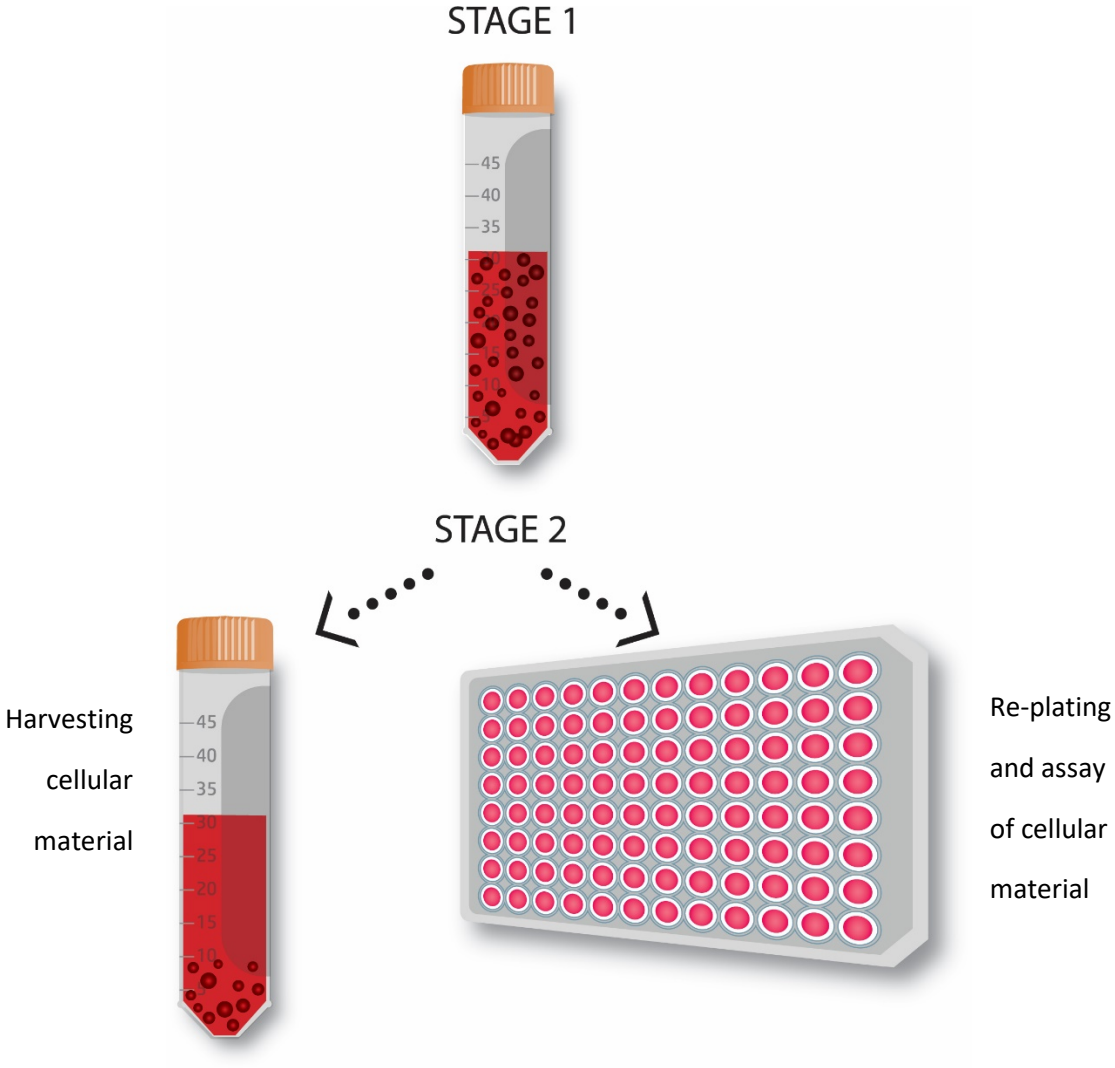

Figure 2. Cellular material can be either harvested or re-plated on low attachment VHP microplates (provided) allowing for further assay experimentation.

Harvesting Cellular Material

If you wish to collect a pellet for protein or nucleic acid analysis, cells should be treated with Happy Cell® ASM Inactivation Solution while in the bioreactors **(Figure 3).** Use the Inactivation Solution as recommended but add a centrifugation step prior to media aspiration to ensure a compact pellet.

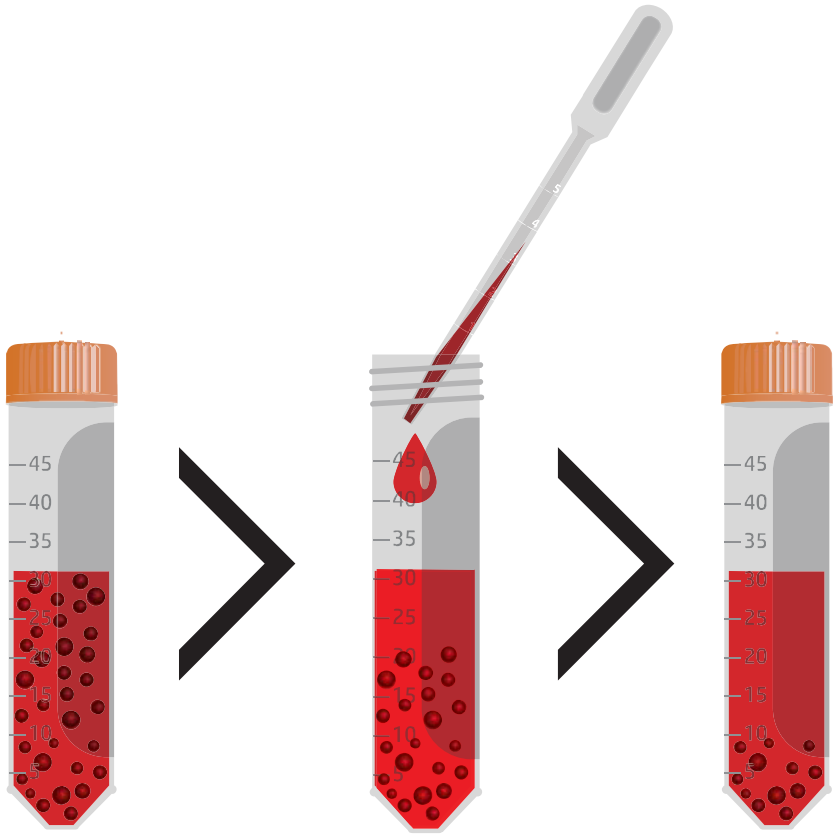

Figure 3. Harvesting cellular material using Happy Cell® ASM Inactivation Solution.

Step 1

When your cultures are ready to harvest, we recommend isolating cellular material by using Happy Cell® ASM Inactivation Solution.

Step 2

Add Happy Cell® ASM Inactivation Solution to your culture and incubate at 37°C for up to 60 minutes.

Step 3

When Cell Suspension has sedimented to the bottom of the Culture Vessel harvest, process and analyze as required.

Using Vale High Performance microplates (VHP)

VHP microplates have been developed to reduce the disadvantageous effects of working with microplates, such as edge effects, evaporation of medium, and the temperature fluctuations that occur with repeated removal of microplates from the incubator.

The plates have low attachment surfaces and used in conjunction with Happy Cell® ASM they offer a number of experimental options. Cells can be seeded directly into VHP plates and micro-tissue formation observed over time or cellular aggregates/micro-tissues can be pre-grown in bioreactor tubes and then dispensed into VHP plates for further experimentation.

VHP plates are produced from high quality, imaging grade materials, so they accommodate micro-tissue culture, maintenance, fixing, washing, labelling and imaging all in the same well.

Safety warnings and precautions

For research use only.

Not recommended or intended for diagnosis of disease in humans or animals.

Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend this product be handled only by persons trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.