Kit #1 – Happy Cell®ASM Trial Kit

Product Information & User Guide

About Kit #1 Happy Cell® ASM

This kit is designed for proof of principle experiments with Happy Cell® Advanced Suspension Medium (ASM). It contains sufficient volume of Happy Cell® ASM and Inactivation Solution to conduct the recommended optimisation experiment – refer to the online "Happy Cell® ASM Preparation, Optimisation and User Guide".

The kit is available in media bases: MEM, DMEM, RPMI 1640.

Catalogue Number: VHCK1

Components

- 2 x 1.5 mL Vials of Happy Cell® ASM supplied as a 4X concentrate
- 0.25 x mL vial of Inactivation Solution (5 mg/mL)
- 1 x VHP low attachment 96 well microtiter plate with lid

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.

Storage and Expiry

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

Ship at ambient temperature.

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Preparation and Use

The Happy Cell® ASM Kit #1:

Stage 1

3D cell culture. Involves the use of VHP low attachment 96 well microtiter plate.

- Wash and re-suspended cells in the desired density in Happy Cell® ASM. The recommended use for long term culture in microtiter plates is 0.05-0.15 mL of premixed Happy Cell® media
- 1X dilution is suitable for most applications unless otherwise stated
- **Volumes etc. can be calculated using the "Happy Cell® ASM Quick Calculator"**
- **We recommend a cell density of 1X10^6 unless otherwise stated**
- Transfer cells to wells of microtiter plate
- Incubate at 37°C at 5% CO_2
- Monitor your culture using an inverted microscope

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 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 **ensuring that the serum and supplement levels are always maintained:**

I. Topping up the level of Happy Cell® ASM in the cell culture vessel/microtiter plate. 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 e.g.Figure 1 below.

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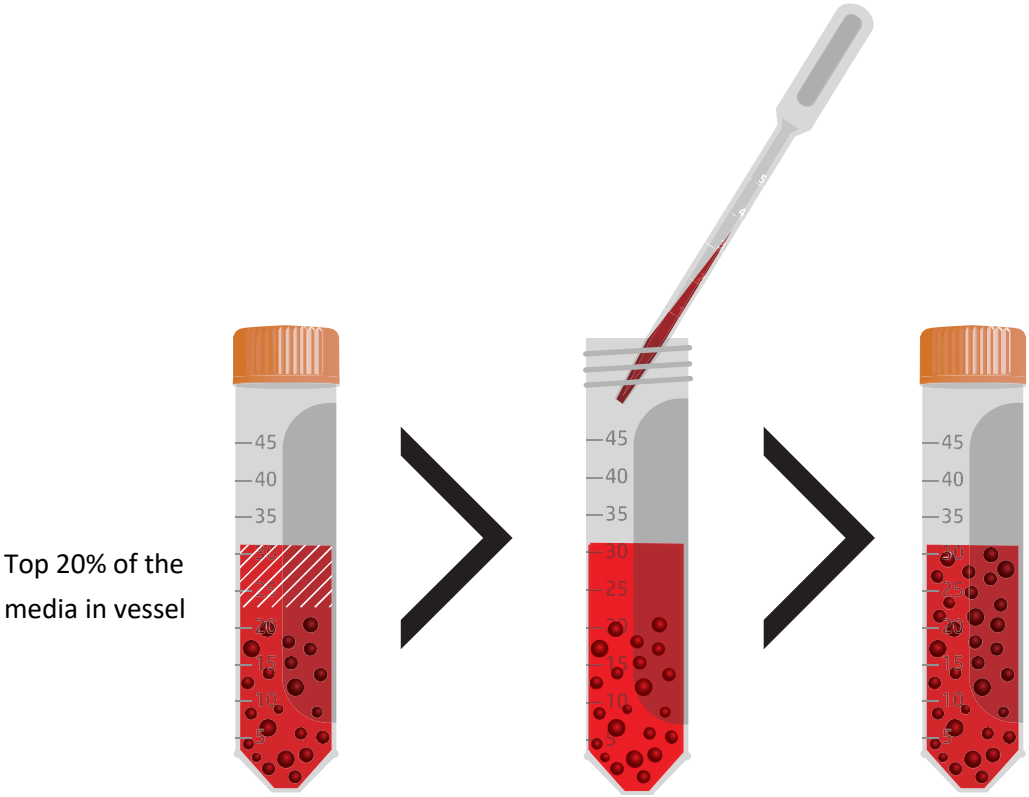

Figure 1. Principle of media replacement in tubes or microtiter plate using Happy Cell®ASM

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 clear of cellular material.

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.

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Stage 2

Harvesting Cellular Material

If you wish to collect material for protein or nucleic acid analysis, cells should be treated with Happy Cell® ASM Inactivation Solution while in culture vessel e.g. microtiter plate or bioreactor tube **(Figure 2).** Use the Inactivation Solution as recommended if necessary, add a centrifugation step prior to media aspiration to ensure no material is lost.

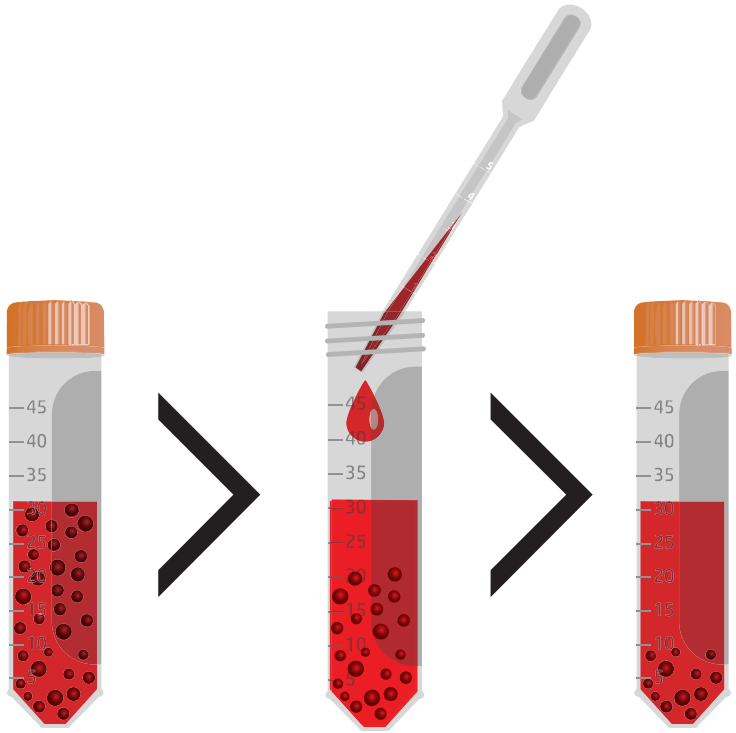

Figure 2. Principle of harvesting cellular material using Happy Cell® ASM Inactivation Solution.

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.

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Using Vale High Performance microplates (VHP)

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

VHP 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.

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