Happy Cell® ASM
Preparation, Optimisation & Use

User Guide

About Happy Cell® ASM

Happy Cell® ASM is a liquid matrix with a multitude of uses. When mixed with cell culture medium, it supports cell growth in suspension, enabling the formation and growth of 3D multi-cellular structures (spheroids), the expansion and growth of single cells, and enhances the viability of primary cells. When no longer required this suspension system can be inactivated, thereby simplifying sample preparation, downstream processing and cell harvesting.

You can choose from the following media bases: MEM, DMEM, RPMI. Other media bases may be available on request.

Use of low adhesion cell culture vessels is recommended.

Catalogue Number: VHCME; VHCDM; VHCRP

Components

- 1 x 25 mL bottle of Happy Cell ASM supplied as a 4X concentrate

Contains Penicillin 10,000 IU/ml and Streptomycin 10,000 µg/ml (P/S) at a final concentration of 1%. We recommend maintaining this concentration throughout your experimental work.

Contains Phenol Red (pH indicator).

Additional Items Required

- Cell culture medium of your choice, e.g. RPMI, DMEM etc.
- Desired cell culture additives, e.g. Fetal Bovine Serum (FBS), L-Glutamine
- Penicillin, Streptomycin
- Low attachment microplates or cell culture vessels/bioreactors, e.g. Vale High Performance low attachment microplates

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

- Eppendorf tubes, pipettors, tips etc.

Please note: Happy Cell® ASM contains Penicillin Streptomycin at a final concentration of 1%. We recommend maintaining this concentration throughout your experimental work.

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

- Happy Cell® ASM is supplied at a 4X concentration and is readily diluted to the required working concentration with standard cell culture medium and desired additives.
- A 1X concentration and seeding density of 0.5 – 1 x 10^6 cells/ml is appropriate for most applications.
- Suggested dilution protocols for three (3) concentrations are outlined in the table below – **Table 1.**
- The 4X concentration may be slightly viscous. If difficulties occur during pipetting, use blue tips (for 1ml pipettor, with larger hole). If problems persist, enlarge hole by cutting a few mm off the tip.
- The following sample dilution options are based on a final volume of 2.5 ml working solution and assume 10% Fetal Bovine Serum (FBS) and 1% L-Glutamine.

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

Final Happy Cell® ASM concentration	Happy Cell® ASM (4X) (ml)	FBS (ml)	L-Glutamine (ml)	MEM (including 1% Penicillin and Streptomycin) (ml)	Final working volume (ml)
3X	1.875	0.25	0.025	0.35	2.5
2X	1.25	0.25	0.025	0.975	2.5
1X	0.625	0.25	0.025	1.6	2.5
0.5X	0.313	0.25	0.025	0.912	2.5

Table 1. Example dilution series to determine the optimum concentration of Happy Cell®ASM with cell-based experiments.

Use

- Collect cells by centrifugation.
- Re-suspend in Happy Cell® ASM prepared to the required working concentration.
- Plate out in low adhesion culture vessels.

Maintenance of Cell Cultures

- Happy Cell® ASM has been designed to sustain cells in long-term culture.
- To ensure healthy growth kinetics cultures should be regularly fed.
- As metabolic activity and nutrient uptake differs widely between cell types monitor the colour of the phenol red pH indicator in the media.
- Replenish media with the same working concentration of Happy Cell® ASM.

Use either one of two methods:

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

   Or

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

2. Remove a small volume of medium from the surface of the liquid (~20% total volume) and replace with fresh medium. Make sure not to disturb the cells (Figure 1 below).

Figure 1. Media replacement using Happy Cell® ASM in either bioreactor tubes or microtiter plates.

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 of cells.

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.

Version – V2.1 – 16 Jan 2019

T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

Analysing Cell Cultures Grown in Happy Cell® ASM

Cell cultures can be analysed, quantified, stained and/or processed using standard sample preparation and labelling methodologies. Cellular recovery can be facilitated using Happy Cell® ASM Inactivation Solution (Catalogue Number VHCIS).

Optimisations

Depending on the cell line in question, spheroids will grow at different rates and to different sizes and densities. A higher concentration of Happy Cell® ASM may be required to support larger spheroids and maintain cellular suspension. If you experience any problem optimise Happy Cell® ASM concentration and/or cell seeding density for your cell type by following the optimisation procedure outlined below.

We recommend carrying out the optimisaton in a 96-wellplate using three Happy Cell® ASM concentrations, 3X, 2X and 1X, and cell cultures at six different seeding densities. A final volume of 100ml/well is assumed.

Preparation of cell dilution series

For each Happy Cell® ASM concentration to be tested prepare serial dilutions as follows:

- Prepare 6 Eppendorf tubes and label 1 – 6
- Add 500ml of Happy Cell® ASM to tubes 2 - 6
- Count cells as usual, place 4 x 106cells in tube 1, centrifuge and remove supernatant
- Re-suspend in 1 ml Happy Cell® ASM at the concentration to be tested (final concentration of 4 x 106cells /ml)
- Serially dilute by taking 500ml of tube 1 and add to tube 2, mix, add 500ml of tube 2 and add to tube 3, mix, etc
- Plate cultures (100ml/well) as shown in Figure 2 below.
- Incubate at 370C and monitor cell growth and/or formation of 3D spheroids until desired outcome is achieved. Depending on cell line this may take several days

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

Figure 2. Schematic of a suggested strategy for optimising cell culture using Happy Cell® ASM.

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com

Trouble shooting

Problem	Suggested solution
Cells attach to culture vessel	1. Make sure low adhesion culture vessels are used. 2. A higher concentration of Happy Cell® ASM may be required. Optimise Happy Cell® ASM concentration and/or cell seeding density. 3. Layer cell suspension on top of Happy Cell® ASM in culture vessel.
4X solution of Happy Cell® ASM is too viscous to pipette	Use blue pipette tips for 1ml pipettor, with large hole. If problem persists, cut a few mm off the tip to enlarge hole.

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.

Version – V2.1 – 16 Jan 2019
Vale Life Sciences© Pty Ltd
T: +61 410867205 | Email: enquiries@valelifesciences.com | www.valelifesciences.com