



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 attachment cell culture vessels are 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



- 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 of Happy Cell ASM and seeding density of $0.5 - 1 \times 10^6$ cells/ml is appropriate for most applications.
- Happy Cell® ASM contains Penicillin 10,000 I.U./mL and Streptomycin 10,000 (µg/mL).
- Any media used for diluting Happy Cell® ASM should contain antibiotics at same concentration.
- 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.
- **Volumes can also be calculated using the “Happy Cell® ASM Quick Calculator”.**

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.
- Monitor the colour of the phenol red in media. Replenish if colour changes from red to yellow.



- Replenish media using one of the two methods below (**ensuring that serum and supplement levels are maintained**)

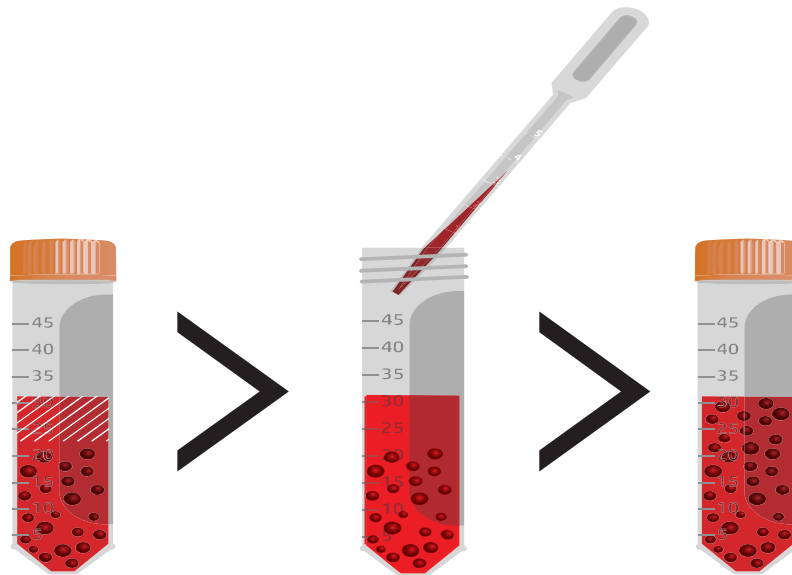
Use either one of two methods:

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
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.

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 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.



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 optimisation in a 96-wellplate using Happy Cell® ASM concentrations, 1X and 2X and using cells at a range of seeding densities from 1×10^2 to 1×10^6 .

Trouble Shooting

Problem	Suggested solution
Cells attach to culture vessel	<ol style="list-style-type: none">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.