# Fixing and Labelling of Single Cells and Spheroids Cultured in Happy Cell® ASM

## User Guide

## About Happy Cell® ASM

**Happy Cell® ASM permits the labelling and staining of both live and fixed cells.**

**Happy Cell® ASM, as with normal cell culture media, will permit unimpeded diffusion of chemical dyes and other reagents.**

**Catalogue Number:** VHCXX

## Labelling Live Cells

To label live cells use reagents as per manufacturer's instructions. Inactivate when required (e.g. for microscopy applications).

## Labelling Fixed Cells.

Fixed end point applications may require some or all the following preparation steps:

### A. Washing

- Washing can be performed either by sedimentation under gravity or by centrifugation depending on the structural integrity of the spheroids.
- For delicate spheroids we recommend sedimentation.
- For robust spheroid structures we suggest low speed centrifugation.

## Steps for Sedimentation under Gravity

**Perform a PBS wash by:**

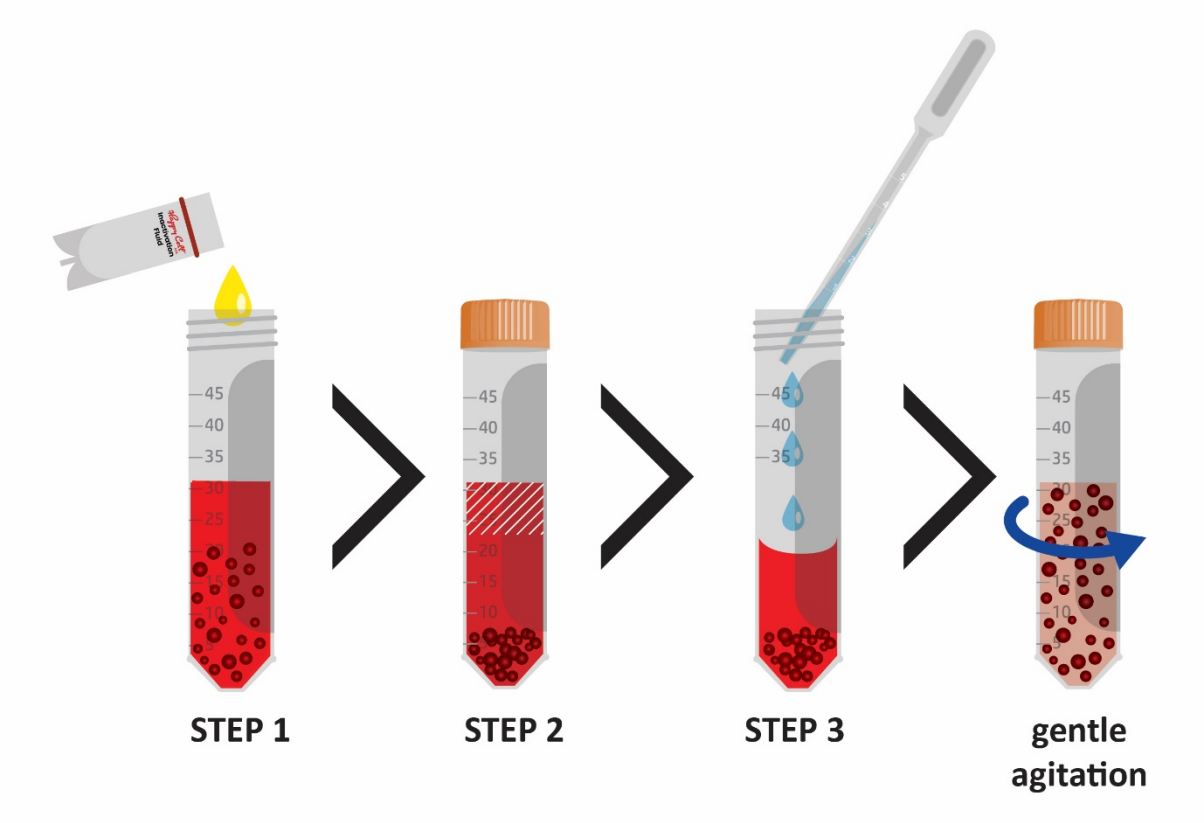
**Step 1** Treat suspension culture with Inactivation Solution as recommended (see Happy Cell® ASM Inactivation Solution – User Guide).

**Step 2** After inactivation, when the spheroids have accumulated at the base of the culture vessel, remove all but 75% of the media. Aspirate from the surface of the media so as not to disturb the cellular material below.

**Step 3** Perform a PBS wash by replacing the removed media with the same volume of PBS and gently mixing or agitating to re-suspend the cellular material.

**Step 4** Incubate at room temperature for 20 mins to allow cells to sediment once more.

**Repeat Steps 3 and 4 another two times. I.e. a total of 3 washes.**



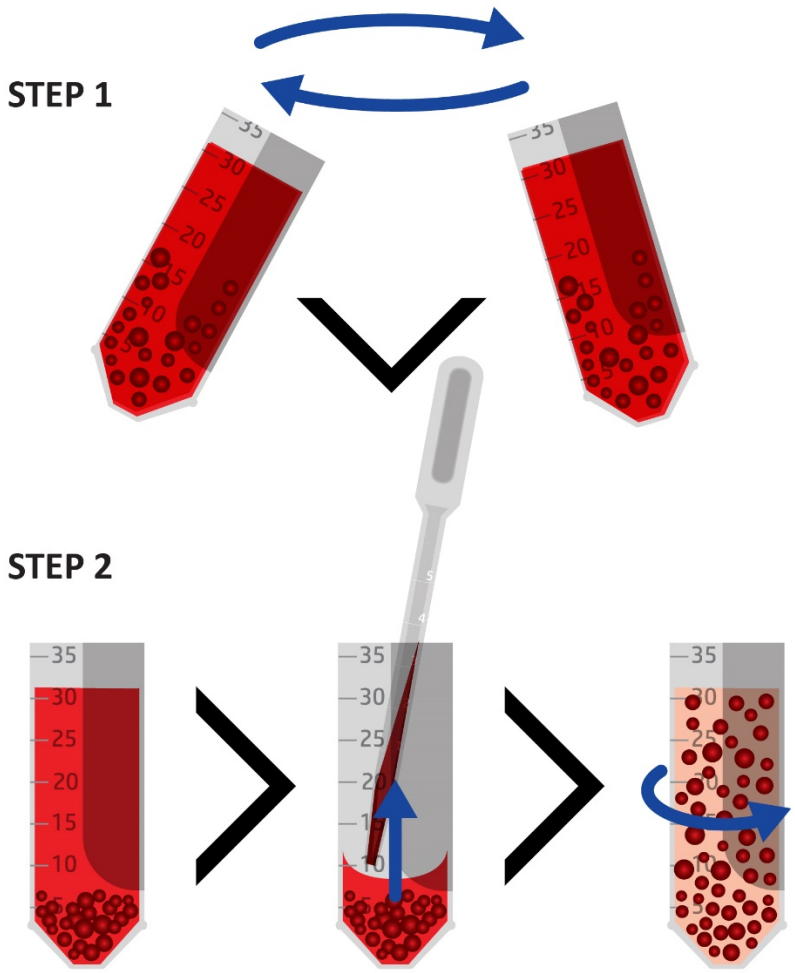
## Steps for low speed centrifugation

**Step 1** Centrifuge at low speed (20 x g for 5 minutes).

**Step 2** Wash gently and carefully remove the added PBS from the surface of the liquid as before. If using centrifuge tubes aspirate to just above the pellet.

**NOTE:** Preliminary tests have indicated that spheroids will sediment readily at 20 x g. Do not spin at higher g values as this can compromise the integrity of the structures. This step may require some optimisation.

**Repeat Steps 1 and 2 another two times. i.e. a total of 3 washes.**



### B. Fixing

- Fix with paraformadelhyde (PFA) to a final concentration of 2%.
- Incubate overnight at room temperature.

  **Note:** We recommend a treatment time of 24 hours for fixation stage.

### C. Permeabilising

- Permeabilise with 0.5% Triton X-100 for 20 minutes at room temperature.
- Wash three times again as per instructions above

  **Note:** If paraformaldehyde and/ or Triton X 100 is not suitable for your experimental procedures, we recommend using your chosen fixative and permeabilisation agents at the standard working concentration for your cell type.

### D. Labelling

- Label with your choice of stains or antibodies as per manufacturer's instructions. If conducting immunofluorescence add in a block step prior to primary antibody addition.

**Note: As with all experimental procedures it is strongly recommended that you first test and optimise your fixation and permeabilisation procedures prior to performing large scale or important studies.**

## 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.