Fixing and Staining of Spheroids Cultured in Happy Cell ASM

Product Information Sheet

Catalogue number:VHC XX

Happy Cell ASM permits the labelling of both live and fixed cells. For labelling live cell cultures we recommend following the manufacturer's instructions. Staining of live cells can be performed either when cells are in suspension or after sedimentation. For sedimentation and/ or recovery of cellular material we recommend using Happy Cell ASM Inactivation Solution (see product information sheet). Happy cell ASM, as with normal cell culture media, will permit unimpeded diffusion of chemical dyes and other reagents. We do recommend gently mixing cultures when adding experimental reagents such as drugs, dyes, growth factors etc.

The following protocol outlines the steps required to wash, fix, permeabilise and label both single cells and spheroids cultured in Happy Cell ASM.

1. Treat suspension culture with Inactivation Solution as recommended (see protocol).
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.
3. Perform a PBS wash by replacing the removed media with the same volume of PBS and gently mixing or agitating to resuspend cellular material. Incubate at room temperature for 20 minutes to allow cells to sediment once more. Alternatively, centrifuge at low speed (20 x g for 5 minutes). After 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. We have found that spinning at higher g

Unit 3053, Level 3, Translational Research Institute, 37 Kent Street, Woolloongabba, QLD 4102, Australia.
T: +61 410867205 Email: enquiries@valelifesciences.com www.valelifesciences.com

values can compromise the integrity of the structures. This step will require some optimisation on a case by case basis.

4. Repeat step 3 twice more (total of 3 washes).
5. Fix using paraformaldehyde (PFA) at final a concentration of 2%. Incubate overnight at room temperature.
6. Perform 3 x PBS washes as before.
7. Permeabilise with 0.5% Triton X-100 for 20 minutes at room temperature.
8. Repeat step 6.
9. Label with your choice of fluorophores or antibodies as per manufacturer's instructions. If conducting immunofluorescence add in a block step prior to primary antibody addition.

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. We also recommend a treatment time of 24 hours for fixation stage. 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.

Unit 3053, Level 3, Translational Research Institute, 37 Kent Street, Woolloongabba, QLD 4102, Australia.
T: +61 410867205 Email: enquiries@valelifesciences.com www.valelifesciences.com