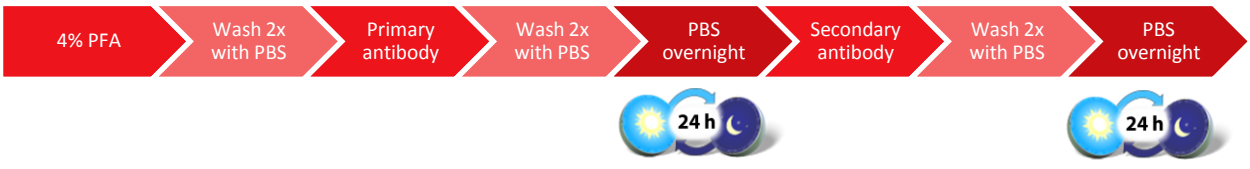
Immunostaining of samples within CellDirector® 3D

IMPORTANT:
When staining your sample within CellDirector 3D it is essential that the outlet tube is kept in place. Do not remove the outlet tube!

1. After the experiment, fix the cells using 4% paraformaldehyde (PFA). Add the fixation solution by slowly pipetting 150 µl of PFA into the outlet tube. Incubate between 1-10 min to fix your sample.
2. Wash away the fixation solution with 2 x 150 µl PBS, by slowly pipetting via the outlet tube.
3. Empty the channels by slowly pipetting air into CellDirector 3D via the outlet tube.
4. Add 10 µl of primary antibody with a p10 pipette via the cross-shaped slit.
   Incubate for 1 hour.
5. Wash with 2 x 150 µl PBS, by slowly pipetting via the outlet tube.
6. Immerse the CellDirector assay in a beaker filled with PBS and place the beaker on a shaker. Incubate at 4°C for ~24 hours.
7. Retrieve the CellDirector assay and add 10 µl of secondary antibody with a p10 pipette via the cross-shaped slit. Incubate for 1 hour.
8. Wash with 2 x 150 µl PBS, by slowly pipetting via the outlet tube.
9. Again immerse the CellDirector assay in a beaker filled with PBS and place the beaker on a shaker. Incubate at 4°C for ~24 hours.
10. Retrieve the CellDirector assay, wipe using a paper tissue and examine the sample by microscopy.



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