Mimetix® Protocol: Cell Visualisation

Imaging cells within the Mimetix scaffold with fluorescent or confocal microscopy

Confocal microscopy is a useful tool to obtain high-resolution images of cells within the Mimetix® scaffold and to investigate their depth profile. But cells can be easily observed using a "classic" fluorescence microscope too. In the example protocol in the next paragraph, nuclei are stained with TO-PRO® 3 and F-actin with Alexa Fluor® 488 Phalloidin . The Mimetix scaffold used here is a bespoke product and has Rhodamine 6G incorporated into the fibres. Please contact us if this could also be of interest for your research.

For a typical experiment using Mimetix® in a 12-well plate format, prepare a cell suspension at a concentration of 10^5 cells/mL for short experiments (1-3 days) or 5 * 10^4cells/mL for longer experiments (up to 7 days) and add 1 mL into each well. Allow cells to adhere for 24 hours or overnight.

- To fix the cells in the scaffolds, aspirate medium and add 0.5 mL pre-warmed 4% paraformaldehyde in PBS to each well.
- Incubate for 15-30 min, then aspirate paraformaldehyde, wash 2x with PBS and add 0.5 mL 0.1% Triton X-100 in PBS to each well to permeabilize cell membranes (only necessary when performing Alexa Fluor® 488 Phalloidin staining).
- Incubate for 5 min, then aspirate Triton X-100, wash 2x with PBS and add 0.5 mL 0.5% BSA in PBS to each well to act as a blocking agent (only necessary when performing Alexa Fluor® 488 Phalloidin staining).
- Incubate for 30-60 min, then aspirate 0.5% BSA in PBS, wash 2x with PBS and add 200 µL Alexa Fluor® 488 Phalloidin working solution into each sample.
- Incubate for 30-60 min, then aspirate Alexa Fluor® 488 Phalloidin, wash 2x with PBS and add 1 mL TO-PRO® 3 working solution (1:10,000 in PBS) to each dish.
- Incubate for 10 min, then aspirate TO-PRO® 3, wash 2x with PBS and 1x with water to prevent the formation of salt crystals.
- Remove scaffold discs from wells and place on a glass slide, cell-seeded side facing up. Add a drop of mounting medium, place cover slip on top (dry off excess mounting medium with paper towel) and seal edges with nail varnish.
- Image stained cells within Mimetix® by exciting Alexa Fluor® 488 Phalloidin at 488 nm using an Argon laser and detecting at 500-550 nm, Rhodamin 6G-labelled fibres at 543 nm using a He-Ne laser and detecting at 550-615 nm, and TO-PRO® 3 at 633 nm using a He-Ne laser and detecting at 650-710 nm.

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