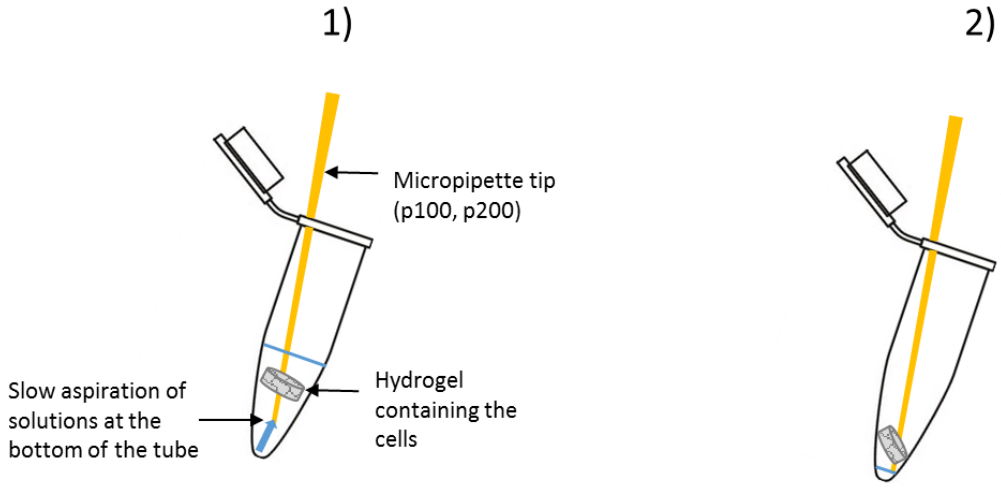
Immunofluorescent markers

Protocol for immunofluorescent markers

BIOMIMESYS® allows cell culture in 3 dimensions and their observation by fluorescence microscopy without changing the labelling and observation protocols commonly used.
It is possible to place the hydroscaffold in a test tube for fluorescence labelling. This allows washing and aspiration of the hydroscaffold safely (see diagram below) and requires small quantities of antibodies/probes.



Fixation

- Place the hydroscaffold containing cells in a microtube with the use of a fine forceps.
- For a hydroscaffold, we recommend washing with 500µL and incubation with 200µL antibodies.
- Rinse with PBS.
- Fix the cells with 4% paraformaldehyde (PAF) in PBS for 15 minutes at room temperature.
    - Rinse the cells three times with PBS.
    - The samples may be stored in PBS at 4°C for several weeks.

⚠

- *Remember to take out paraformaldehyde from the freezer and heat to 37°C if white precipitate is present.*
- *If the biological sample is thick or dense, increase the fixing time in PAF to 30-45min.*

Permeabilization

- Incubate the cells in 0.5% Triton solution for about 6 minutes to make the cell membrane more permeable to allow the entry of the antibody into cells.
- Rinse 3 times with PBS.

Blocking

- Incubate the cells in PBS + 1% BSA solution for 1 hour at room temperature or overnight at 4°C.
- Rinse 2 times with PBS + 0.1% BSA.

Marking

- Add the primary antibody (concentration to be adapted depending on the antibody) and incubate for 1 hour at room temperature in PBS + 0.1% BSA.
- Rinse 3 times with PBS + 0.1% BSA.
- The primary antibodies can be detected using anti-IgG secondary antibody (depending on the source of the primary antibody: mouse, rabbit or sheep) coupled to a fluorophore (green or red) and by adding DNA intercalating agents such as DAPI or Hoechst (1ug/ml).
- Incubate at room temperature for 1hours 30min away from light.
- Rinse 1 time with PBS + 0.1% BSA.
- Rinse 2 times with PBS.

Observation

- Place the sample on the glass slide with a drop of PBS (careful not to dry out the sample during observation; add about 20µL of PBS during image acquisition, if needed).
- The samples can be stored for several weeks in a tube filled with PBS at 4°C away from light.

Contact Information

HCS Pharma

hello@biomimesys.com

www.biomimesys.com