# Immunostaining in BIOMIMESYS® *hydroscaffolds™*

**Product:** BIOMIMESYS® 3D Cell Culture Hydroscaffold™

**Format:** 96 well-plate black/clear & 96 well-plate clear

**Context:** BIOMIMESYS® is a unique groundbreaking 3D cell culture technology which associates the behavior of a solid scaffold and of a hydrogel. It provides a cell culture microenvironment reproducing all aspects of human tissues, including matrix architecture, cellular organization, cell-cell and cell-matrix interactions. Depending on the organ, extracellular matrix components and their proportions may vary, allowing a more or less dense and compact cellular environment (different Elastic Moduli, porosities). BIOMIMESYS® matrices are made of Hyaluronic Acid (HA), the main glycosaminoglycan (GAG) of the ECM, collagens and adhesion proteins. Our patented manufacturing process allows to preserve the natural properties of HA and therefore synthesizing proprietary Hydroscaffold™.

Immunofluorescence is a technique which uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualization of the distribution of the target molecule through the sample. It allows to study the properties, functions, interactions and production of chemical components (antibodies / immunoglobulins, toxin, epitopes of proteins such as CD4, antitoxins, cytokines / chemokines, antigens) of cells.

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BIOMIMESYS® allows cell culture in 3 dimensions and their observation by fluorescence microscopy without changing the labelling protocols commonly used. For changing solutions, proceed the same way as for changing medium (see Starting guide). You can discard all the solution before adding a new one, or discard half of it and add your solution at a 2X concentration. If you cannot concentrate the solution, increase the time of incubation. Perform the same steps as usual:

## 1. Fixation

- Rinse 3 times with 200µL per well of prewarmed PBS.
- Fix the cells with 50µL per well 4% paraformaldehyde (PAF) for **at least** 15 minutes at room temperature. If the biological sample is thick or dense, you can increase the fixation time up to 30 minutes.
- Rinse 3 times with 200µL per well of PBS.

## 2. Permeabilization

- Permeabilization time must be **longer** compared to 2D cultures. Usually, we permeabilize with 50µL per well of Triton X-100 at 0.1% (weight/volume) during 30 minutes at room temperature.
- Rinse 3 times with 200µL per well of PBS.

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## 3. Blocking

- Incubate the cells in your usual blocking solution during 1 hour at room temperature or overnight at 4°C. For example, we use 200µL/ per well of a 1% BSA solution in PBS.

## 4. Immunostaining

Perform your immunostaining protocol as usual. The minimal volume for the staining is 50µL per well. For example:

- Incubate at least 2 hours at room temperature or overnight at 4°C with 50µL per well of your primary antibody in a 1% BSA solution.
- Rinse 3 times with 200µL per well of PBS.
- Incubate at least 1 hour in dark at room temperature with 50µL per well of your secondary antibody and/or other dyes such as DAPI, in a 1% BSA solution.
- Rinse 3 times with 200µL per well of PBS.

## 5. Observation

- You can observe your cells under a microscope directly into the plate or you can transfer the *hydroscaffolds*™ onto a glass slide using curved end-tweezers.
- When using a confocal microscope: the black plates in which BIOMIMESYS® is provided are compatible with confocal microscopy. If you have transparent plates, you can transfer the *hydroscaffolds*™ into a plate compatible with microscopy or onto a glass slide.
- Buffer should be discarded before doing microscopy to prevent the *hydroscaffolds*™ from floating.
- When looking for your spheroids/cells, we recommend to take pictures every 100µm on a large range (600 to 1000µm). Once you have determined the location of your spheroids, you can take pictures on a smaller range (usually 300µm maximum) every 1-20µm according to the size of your objects and the precision you want.
- If the cells are blurred, *hydroscaffolds*™ can be flipped using curved end-tweezers.

## Contact Information

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