Preparation of BIOMIMESYS® *hydroscaffolds*™ cell culture samples for histological analysis

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™.

Histological techniques aim at evaluating the morphology and structure of cells, tissues, and organs under the microscope. BIOMIMESYS is compatible with such techniques, allowing to observe the organization of cellular structures (cell aggregates, organoids) obtained within BIOMIMESYS®. This protocol describes the different steps of classic histology workflow, i.e. how to perform fixation, inclusion of samples, staining with hematoxylin-eosin-safran (HES) and immunohistochemistry in BIOMIMESYS®.

BIOMIMESYS® allows cell culture in 3 dimensions and their observation microscopy, without changing the labelling protocols commonly used. Perform the same steps as usual:

1. Fixation

- Fix the samples with A.F.A (Alcohol (usually ethanol), Formalin, Acetic acid) histological fixative mixture or freeze them at -80°C by using OCT Embedding matrix, for **24 hours**

2. Paraffin inclusion and microtome slicing

- Dehydrate the samples successively in :
  - Ethanol 100° for 1 hour
  - Acetone: 4 times for 1 hour each
  - Acetone for 1h15
  - Xylene: 3 times for 1 hour each

- Include the samples a first time in paraffin at 56°C for 1 hour, and a second time in paraffin again for more than 1 hour

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- Cut the samples (5 µm-thick slices) using a microtome
- Stick the slices on fat-free and uncoated slides, using albumin-glycerol

3. Protocol for hematoxylin-eosin-safran (HES) staining

- Remove the paraffin the using OTTIX and OTTIX shaper (Diapath)
- Staing the slices successively with:
    - Harris hematoxylin for 6 minutes
    - Eosin G for 1 minute
    - Safran for 3 seconds
- Dehydrate the samples in OTTIX/OTTIX shaper
- Mount the sample with mounting medium (Entellan®, Millipore)

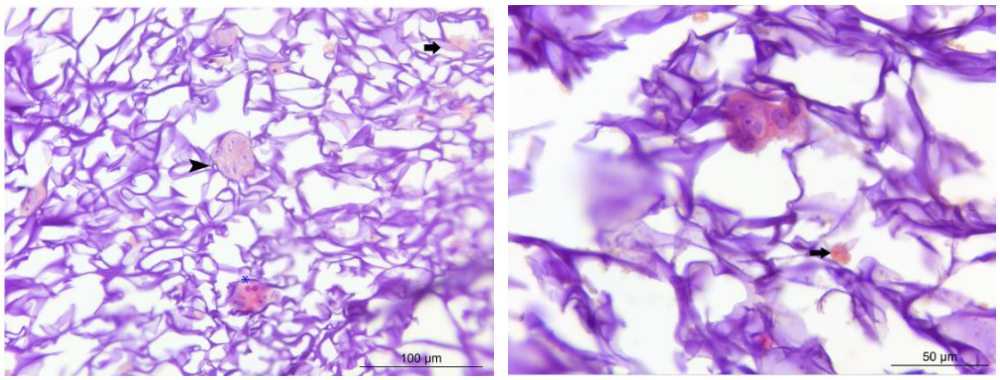

Figure 1: HES staining on a slice of BIOMIMESYS containing cells (matrix in purple ; cytoplasm in pink, nuclei in purple)

4. Protocol for immunohistochemistry

- Remove the paraffin the using OTTIX and OTTIX shaper (Diapath)
- Unmask the antigens with citrate buffer at 10 mM (pH 6) for 12 minutes (or only 6 minutes if the slices come off from the slides)
- Heat the slices the citrate buffer in a microwave oven Chauffer les coupes avec le tampon citrate au four à micro-onde
- Incubate the samples overnight with the primary antibody at 4°C
- Inhibit the endogenous peroxidases with hydrogen peroxide (30 volumes) diluted in PBS-BSA at 3%
- Incubated the slices for 45 minutes with the secondary peroxidase-linked antibody
- Add diaminobenzidine (DAB) (Dako, SK3468) for revealing the antibodies-antigens complexes by a brown staining
- Stain the slices with Mayer's hematoxylin
- Mount the slices between slide and cover glass in aqueous medium

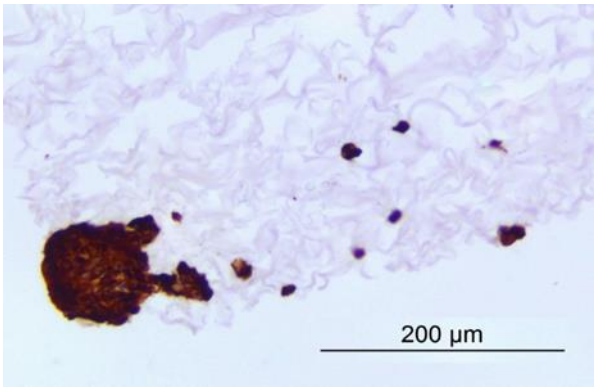

*Figure 2: Staining of BIOMIMESYS samples containing fibroblasts. BIOMIMESYS hydroscaffold appears in pink following staining with Mayer's hematoxylin, and the fibroblasts were stained using anti-vimentin antibody (*M0725, lot 00038159, Dako)

Contact Information

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