Preparation of samples for histological labelling

It is possible to make histological sections as well as perform Hematoxylin-Eosin-Saffron staining and/or immunohistochemical reactions on BIOMIMESYS®.

This protocol can be used with any cell type grown in BIOMIMESYS®. It uses standard reagents for making a histological section but also more specific reagents such as OTTIX and OTTIX shaper (DiaPath) and Entellan® (Millipore).

➢ Sample Fixation

- Fix the samples using alcohol, formaldehyde and acetic acid (4%)
- Fix these samples for 24h at an ambient temperature.

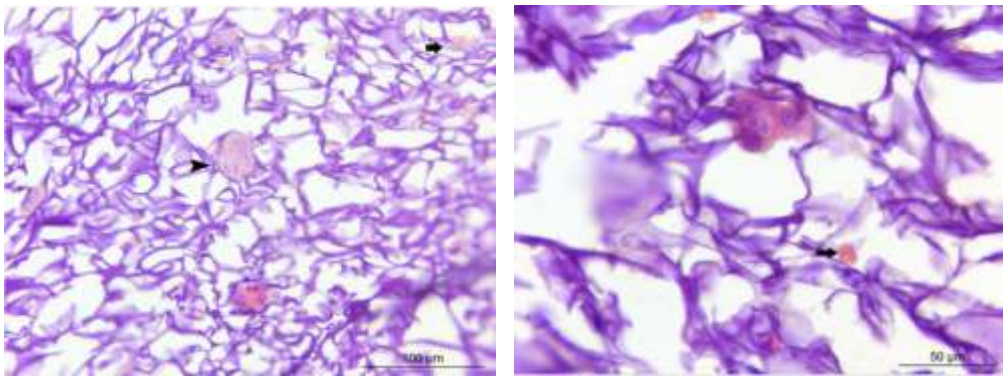
➢ Paraffin cuts

- Dehydrate the samples in successive ethanol, acetone and xylenes washes
    1. Absolute ethanol for 1h.
    2. 4 washes in acetone for 1h each.
    3. 1 wash in acetone for 1h15min.
    4. 3 washes in xylene for 1h each.
- Embed the samples in paraffin at 56 ° C for 1h and repeat to ensure all solvent is removed. hour.
- Cut into 5 µm slices using a microtome.
- Mount the sections onto untreated, degreased slides using glycerine albumen.

➢ HES Staining (Haematoxylin-Eosin-Saffron)

- Deparaffinize the sections in OTTIX and OTTIX shaper (DiaPath).
- Stain these sections successively with :
    1. Harris' hematoxylin for 6 minutes.
    2. Eosin G for 1 minute.
    3. Saffron for 3 seconds.

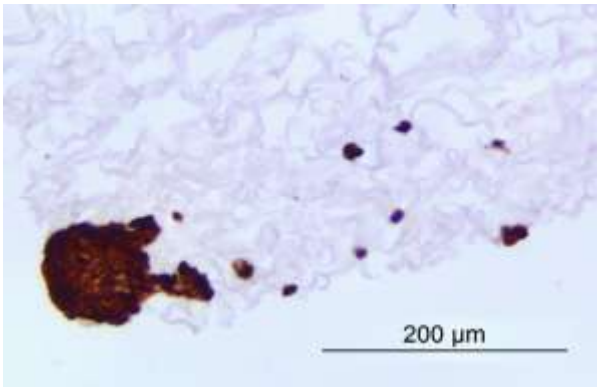
- Dehydrate in the OTTIX/OTTIX shaper.
- Mount using a mounting medium (Entellan®, Millipore).



HES staining performed on a section of BIOMIMESYS® containing cells (matrix: purple; cytoplasm: pink; nuclei: purple)

➢ Immunohistochemistry

- Deparaffinize the sections in OTTIX and OTTIX shaper (DiaPath).
- Uncover the antigenic sites using 10 mM citrate buffer (pH 6) for 12 minutes (or 6 minutes if the cuts come off).
- Heat the sections with the citrate buffer in a microwave oven.
- Incubate the sections overnight with the primary antibody at 4 ° C.
- Inhibit endogenous peroxidase by using 5% hydrogen peroxide (30 vol), diluted in 3% PBS-BSA.
- Incubate the sections for 45 minutes with secondary antibody coupled to peroxidase.
- Add diaminobenzidine (DAB) [Dako, SK3468] to reveal the antigen-antibody complexes as brown markings.
- Stain the sections using Mayer's hematoxylin.
- Mount the sections in the aqueous medium between slide and cover slip.



Staining
BIOMIMESYS® with Mayer's haematoxylin and marking fibroblasts with an anti-vimentin antibody (M0725, lot 00038159 [Dako])

Contact Information

HCS Pharma

hello@biomimesys.com

www.biomimesys.com