E-Mail: info@phenion.com

Web: www.phenion.com

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COMPETENCE IN SKIN PHYSIOLOGY
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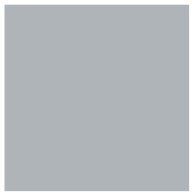
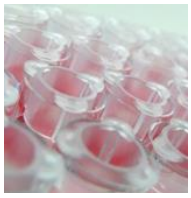
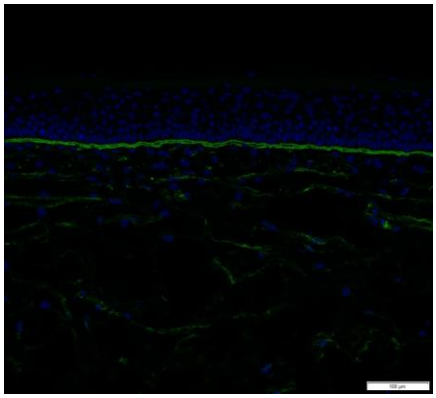
Phenion® FT Skin Model
♦ Immunofluorescent labelling of proteins on the basis of frozen sections







Objective

This Standard Operation Procedure is recommended to identify antigens like proteins and peptides in the cells and tissues of the Phenion® FT Skin Models with specific antibodies directed against said antigens. Best results of immunofluorescent labelling is achieved with frozen sections of the tissue (see: SOP Frozen sections). The process is exemplified for the detection of laminin 5, a basement membrane protein, but can be generally adapted for any antigen/antibody.

Materials

Items	Company	Order-No.
Frozen sections of the tissue	---	---
Glass slides (Superfrost Plus)	VWR	631-0108
Coverslips 24x50mm	Carl Roth	1871
DAKO fat pen	DAKO	S2002
Acetone	e.g. Sigma	1.00012
D-PBS	Gibco	14190-094
Normal Goat Serum	Invitrogen	50197Z
Humid chamber with lid	---	---
DAKO Antibody Diluent	Agilent	S0809
anti-laminin antibody (mouse)	Sigma	L8271
Alexa 488 anti-mouse antibody (goat)	Invitrogen	A11059
DAPI	Sigma	D9542
DAKO Fluorescence Mounting Medium	Agilent	S3023

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Procedure

Fixation of the frozen sections

1. Incubate the frozen sections (either stored at -20° or after air drying at room temperature) in ice-cold acetone (-20°C) for 10 minutes.

2. Let air dry the sections for 5 min afterwards.

   ❖ *Caution: Starting with this working step do not let the sections become dry because this will generate artefacts!*

3. Encircle the sections on the slide with the DAKO fat pen and transfer the slides in a humid chamber. Sufficient humidity to avoid evaporation of the antibody solutions can be generated by soaking tissue papers in water and placing them onto the bottom of a vessel which can be closed with a lid.

Antigen blocking

1. Apply the blocking solution (10% Normal Goat Serum in D-PBS *or* a ready-to-use solution from a supplier) onto each section until the area within the circle is completely covered. The volume needed to cover the section are ~200 µl each.

2. Incubate the sections for 1h at RT in the humid chamber with closed lid.

Incubation with primary antibody

1. Dilute the primary antibody in DAKO Antibody Diluent. For the anti-laminin antibody a dilution of 1:500 is recommended. For any other antibody please follow the recommendations provided by the antibody producer.

2. Remove the blocking solution from the sections by gently tapping the glass slides.

3. Cover each section with 50 -100 µl antibody solution, until the whole section is completely covered.

4. Close the humid chamber with a lid and place it overnight in a refrigerator at 4°C.

Incubation with secondary antibody

1. Dilute the secondary antibody in DAKO Antibody Diluent. For the Alexa 488-tagged goat-anti-mouse antibody a dilution of 1:200 is recommended. Add 0.1 µl of a DAPI solution (10 mg/ml stock solution in D-PBS) to 100 µl of the 2nd antibody solution each.

2. Remove the primary antibody solution from the sections by gently tapping the glass slides.
3. Wash the slides 3x in fresh D-PBS for 5 min each.

4. Cover each section with 50 -100 µl antibody solution, until the whole section is completely covered.
5. Incubate slides in the humid chamber for 1h at room temperature in the dark.
6. Remove the secondary antibody solution from the sections by gently tapping the glass slides.
7. Wash the slides 3x in fresh D-PBS for 5 min each.
8. For tissue embedding use DAKO Fluorescence Mounting Medium to avoid bleaching of the fluorochrome. Store the slides at 4°C in the dark.