Immunohistochemistry for phospho-myosin light chain 2 in adult murine skin

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Method Article

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Abstract

Phosphorylation of the myosin regulatory light chain 2 is a post-translational modification commonly used to report for myosin ATPase activity and actomyosin contractility. While its use in cell culture has been broadly reported in several studies, data on immunostaining in tissues has been sparse and inconsistent. In this protocol we report a methodology to stain phospho-myosin light chain 2 (pMLC2 Ser19) and double phospho-myosin light chain 2 (ppMLC2Thr18/ Ser19) in adult murine epidermis.

Introduction

Note that myosin activity may vary in different tissues and developmental vs. adult stages. This protocol refers to immunostaining of phosphorylated myosin in adult murine skin.

Reagents

Xylol

Isopropanol

Ethanol

H_2O

Dako Antigen Retrieval Agent pH9 (our recommendation Cat.No. S2367)

Phosphate Buffered Saline^{++} (PBS plus 0.2 mM $CaCl_2$, 0.2mM $MgCl_2$)

Bovine Serum Albumin (e.g. Sigma-Aldrich A7906)

Dako antibody diluent (Cat. No. S3022)

pMLC2 Ser19 (Cell Signaling Technologies #3675) or ppMLC2 Thr18/ Ser19 (Cell Signaling Technologies #3674)

Suited fluorophore-conjugated secondary antibodies (e.g. AlexaFluor-conjugates, Molecular Probes #A21203 donkey anti-mouse, highly cross-adsorbed; #A11036 goat anti-rabbit, highly cross-adsorbed)

DAPI or similar nuclear counterstaining

Mowiol or similar mounting medium

Quick-dry nail polish

Equipment

Boiling cuvette

Pressure cooker

Fat pen (e.g. Dako pen)

Humidified chamber

Glass cover slips, thickness #1-1.5

Procedure

Deparaffinization

Deparaffinize the tissue sections from adult murine skin by submerging the sections in the following alcohol row: xylol for 5 minutes, fresh xylol in a separate container for 5 minutes, isopropanol for 5 minutes, 96% ethanol for 5 minutes, 75% ethanol for 5minutes, 50% ethanol for 5 minutes, and H_2O for 5 minutes.

Antigen retrieval

Retrieve the epitopes by boiling the sections in Dako Antigen Retrieval Agent pH9 (Dako) for 20 minutes inside a boiling cuvette in a pressure cooker.

Allow slides to cool down within the retrieval solution for 1h at room temperature and then wash twice with PBS^{++}.

Blocking

Delimit the tissue sections with a fat pen (Dako pen).

Block unspecific binding sites with 5% BSA in PBS^{++} for 1h at room temperature in a humidified chamber. Ensure that the blocking solution covers the entire tissue section.

Primary Antibodies

Incubate tissue sections with primary antibodies diluted in antibody buffer overnight at 4°C in a humidified chamber. Verify that the antibody solution covers the entire tissue section. Use pMLC2 Ser19

(CST #3675) at 1:200 dilution and ppMLC2 Thr18/ Ser19 (CST #3674) at 1:100 dilution.

Secondary Antibodies

Wash the tissue sections 2x with PBS^{++} for 5 minutes each.

Incubate tissue sections with secondary antibodies and DAPI (2µg/ml) for 1h at room temperature in a humidified chamber in the dark.

Mounting

Wash the tissue sections 2x with PBS^{++} for 5 minutes each.

Briefly wash the tissue sections 1x with H_2O.

Mount tissue sections in Mowiol. Ensure that the Mowiol solution covers the entire tissue section. Remove any air bubbles.

Cover with coverslip.

Let the Mowiol solidify for 24 hours (store dry in the dark at room temperature).

Seal slides with quick-dry nail polish.

Store samples in the cold and dark until further analysis.

Troubleshooting

Use freshly cut tissue sections, and evaluate their quality at a light microscope before staining.

Prepare all solutions freshly.

Proceed with image analysis within a couple of days after mounting.

Take a secondary antibody control sample along to evaluate potential unspecific binding /background.

Specificity could be further verified by immunostaining consecutive tissue sections that have been subjected to alkaline phosphatase treatment to remove phosphorylated epitopes (see separate protocol at Protocol Exchange).

Time Taken

Total: 1.5 days

Deparaffinization: 35 minutes

Antigen Retrieval: 20 minutes boiling plus 1 hour cooling

Blocking: 1 hour

Primary antibody staining: Overnight

Secondary antibody staining/ nuclear counterstaining: 1 hour

Anticipated Results

pMLC2 and ppMLC2 immunostaining should be visible as a mostly cortical signal in the different layers of the epidermis. In the dermis, blood vessels should be strongly positive, whereas fibroblasts may show a moderate signal using this protocol.

References

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