Dephosphorylation of epitopes in murine skin sections

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Method Article

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Abstract

Dephosphorylation of epitopes is a common way of testing phospho-specificity of antibodies. Whereas it is a regularly used protocol for dephosphorylation in cell lysates, its use in immunohistochemistry has been limited. Here we describe a methodology to dephosphorylate adult murine tissues by using Antarctic Phosphatase, an alkaline phosphatase. This protocol is compatible with subsequent immunohistochemistry, making it suitable for validating phospho-immunoreactivity in tissues.

Introduction

Reagents

Xylol

Isopropanol

Ethanol

H_2O

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

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

Antarctic Phosphatase (AP) and AP buffer (50 mM Bis-Tris-Propane-HCl, 1 mM $MgCl_2$, 0.1 mM, $ZnCl_2$,pH 6) (NEB, M0289S)

Bovine Serum Albumin (e.g. SIGMA A7906)

Equipment

Boiling cuvette

Pressure cooker

Fat pen (e.g. Dako pen)

Humidified chamber

Incubator 37°C

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 different 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^{++}.

Epitope dephosphorylation

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

Incubate tissue sections with 25U Antarctic Phosphatase in AP buffer, or with AP buffer only (vehicle control) for 2 hours at 37°C in a humidified chamber.

Blocking

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.

Proceed with standard immunostaining protocols.

Troubleshooting

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

Prepare all solutions freshly.

As a positive control, you may use a reliable phospho-epitope staining previously established in your laboratory.

Some phospho-epitopes may need longer phosphatase incubation time.

Time Taken

Total: 4 hours

Deparaffinization: 35 minutes

Antigen Retrieval: 20 minutes boiling plus 1 hour cooling

Epitope dephosphorylation: 2 hours

Blocking: 1 hour

Anticipated Results

In case of phospho-specific antibodies, phosphorylated epitopes should be detected in the vehicle treated samples (provided the target is indeed phosphorylated in the tissue analysed) but should not be detected anymore after adequate phosphatase treatment.

References

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