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IMMUNOHISTOCHEMISTRY USING SLIDE-MOUNTED PARAFFIN SECTIONS

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All steps in the following protocol are carried out at room temperature unless stated otherwise. Recipes for all solutions (highlighted) in **bold** are included at the end of the protocol.

1. Deparaffinizing and rehydration:

a. Immerse slides in xylene for 10 minutes. Repeat this step again in fresh xylene for 10 minutes. (If required, repeat a third time in fresh xylene for another 10 minutes.)

b. Rehydrate sections by sequentially incubating with 100%, 95%, 80% and 60% ethanol for 5 minutes each.

c. Rinse sections with distilled water three times for 3 minutes each.

2. Antigen retrieval (optional):

a. Transfer slides to a microwave-proof container and cover with **citrate buffer** or **Tris-EDTA (TE) buffer**.

b. Heat in the microwave on medium power for 10 minutes.

c. Allow slides to cool in the **citrate buffer** or **Tris-EDTA (TE) buffer** for approximately 35 minutes.

3. Proteintech antibody incubation:

a. Rinse slides three times with 1x **TBST** for 3 minutes each.

b. Incubate slides with 3% H_2O_2 solution (diluted in distilled water) for 10 minutes to quench endogenous peroxidase activity.

c. Rinse slides three times with 1x **TBST** for 3 minutes each, then rinse slides three times with distilled water for 3 minutes each.

d. Prepare 5% normal blocking serum in 1x **TBST**. The serum should be derived from the same species in which the secondary antibody was raised. Block the sections for 1 hour. (Alternatively, use 5% BSA in 1x **TBST** for blocking if the corresponding serum is not available.)

e. Incubate sections with primary antibody diluted in 1x **TBST** for 1 hour, or overnight at 4°C; the optimal antibody dilution ratio should be pre-determined by experimentation. Set up negative controls by omitting the primary antibody incubation step for one slide per each experimental condition.

f. Following primary antibody incubation rinse slides three times with 1x **TBST** for 3 minutes each.

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4.

Signal detection:

Proteintech routinely uses EnVision Kit reagents (Dako) for this step.

a. Apply sufficient peroxidase labeled polymer and incubate for 30 minutes.
b. Rinse slides three times with 1x **TBST** for 3 minutes each.
c. Prepare an appropriate volume of substrate solution prior to use by mixing one drop of Liquid DAB plus chromogen immediately with 1 ml of substrate buffer. Apply the substrate carefully and incubate for 5–10 minutes till a brown color develops.
d. Rinse sections gently with sufficient distilled water.

5.

Hematoxylin counterstaining (optional):

a. To stain nuclei, immerse slides in a bath of hematoxylin for 3 minutes.
b. Rinse slides gently with distilled water.
c. Transfer slides into a 1% HCl, 99% ethanol solution for 10 seconds; transfer to distilled water immediately.

6.

Dehydration and mounting:

a. Immerse slides sequentially into 60%, 80%, 95% and 100% ethanol baths for 5 minutes each.
b. Immerse slides in xylene for 5 minutes. Repeat this step again in fresh xylene for 5 minutes.
c. Mount the section with sufficient mounting media and cover with a cover slip. Air-dry in a well-ventilated area (e.g. fume hood).

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Solutions

Citrate buffer	For 1000 ml	1x TBS	For 1000 ml
10 mM Trisodium citrate+2H_2O	2.9 g	20 mM Tris-base	2.4 g
1.9 mM Citric acid+H_2O	0.4 g	150 mM NaCl	8.7 g
Adjust pH to 6.0		Adjust pH to 7.6	
Add ddH_2O to 1000 ml		Add ddH_2O to 1000 ml	

Tris-EDTA (TE) buffer	For 1000 ml	1x TBST	For 1000 ml
10 mM Tris-base	1.21 g	1x TBS	999 ml
1 mM EDTA $C_{10}H_{14}N_2Na_2O_8$ +2H_2O	0.372 g	Tween-20	1 ml
Adjust pH to 9.0			
Add ddH_2O to 1000 ml			

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