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Immunohistochemistry with fresh frozen tissue sections

For immunohistochemistry, cryostat sections show improved antigen preservation compared to paraffin sections; however, the morphological details are not so well recognizable.

I. Material:

Tissue sections: The cryosections were cut 5-6 µm thick and mounted on cleaned glass slides. After 15 minutes air drying, the sections were fixed in ice-cold acetone for 10 minutes. The sections were air-dried again for 2-24 hours. Covered in aluminium foil the sections were stored at -20° C.

Laboratory equipment: Microscope, glass cuvettes, pipettes, paper filters, graduated cylinders, funnels, vacuum pump, heating plate, ventilator, humid chamber, Q-tips, Kleenex, grease pen (Dako - Pen), diamond pencil, cover slip, timer.

Buffers and solutions:

10x PBS:

KCl (m.w. 74.6):	1.6g/l
KH_2PO_4(m.w. 136):	2.8g/l
$Na_2HPO_4x2H_2O$ (mw 178):	13.4g/l
NaCl (mw 58.4):	77.8g/l

Dilute 1/10 with distilled water before use, check pH and adjust to pH 7.2 if necessary

Store PBS at room temperature for up to 2 weeks

0.15 M Sodium azide:

Dissolve 4.88g NaN_3 (Fluka 71290) in 500ml PBS pH 7.2.

Store at room temperature for up to 2 weeks.

30% H_2O_2: Hydrogen peroxide (Fluka 95300)

Store at 2-8°C

Acetate buffer: Dissolve 1.4 ml acetic acid (Fluka 45731) in 250 ml A. dest., adjust pH to 4.9 with 10M NaOH.

Store at 2-8°C for up to 2 days.

AEC stock solution: Dissolve 0.4g 3-Amino-9-Ethylcarbazol (Sigma A-5754) in 100ml Dimethylformamid (Fluka 40250).

Store at 2-8°C for up to 4 weeks

AEC substrate: 5ml AEC stock solution
95ml acetate buffer, pH 4.9

Filter through paper filter and add 40µl 30% H_2O_2 (Fluka 95300) immediately before use.

Normal sera (for blocking):

Normal goat serum (NGS), (Gibco BRL)
Normal rat serum ((NRS), (BMA Biomedicals)
Normal mouse serum (NMS), (JacksonImmunoresearch)

Peroxidase labeled antibodies

Peroxidase conjugated Goat-anti-Mouse IgG (H+L)
minimal cross-reaction to human, rat, …
(JacksonImmunoresearch, 115-035-166),
store aliquots at -20°C.

Peroxidase conjugated Goat-anti-Rat IgG (H+L)
minimal cross reaction to human , mouse, …
(JacksonImmunoresearch, 112-035-167),
store aliquots at -20°C.

Peroxidase conjugated Goat-anti-Mouse IgG+IgM (H+L)
minimal cross-reaction to human, …
(JacksonImmunoresearch, 115-035-068),
store aliquots at -20°C.

Peroxidase conjugated Goat-anti-Rat IgG+IgM (H+L)
minimal cross reaction to human, …
(JacksonImmunoresearch, 112-035-068),
store aliquots at -20°C

Mayer's Haematoxylin:

"Mayer's Hemalum" for nuclear staining (Fluka 51275); filter before use.

Royalmount: Royalmount, (BMA Biomedicals, T-3402)

DPX-Mountant: Mounting medium for tissue sections (Fluka 44581)

II. Procedure:

1. Remove the slides from the freezer and take away the aluminium foil after five minutes. Dry the sections for 30 minutes in an air stream. (Ventilator, do not heat)

2. Mark slide with diamond pen or pencil, depending on slide.

3. Circle tissue sections with Dako-Pen
   (to keep antibody solutions apart).

4. Dip slide 1x5 minutes in PBS, pH 7.2.

5. Blocking of endogenous peroxidase: Mix 100ml 0.15M Sodium azide and 0,5 ml 30% H_2O_2 in cuvette, place sections in holder, incubate for 20 minutes at room temperature. Rinse 1x with PBS and incubate for 1x5 minutes in PBS, pH 7.2 (100ml cuvette)

Peroxidase is a naturally occurring enzyme that is found in many tissues. This step serves to suppress peroxidase activity that is not specifically associated with the detection antibody.

6. Blocking with serum: Coat entire tissue sections with 10% NGS in PBS, incubate for 30 minutes at room temperature in a humid chamber.

This step serves to block non-specific binding of the antibody to the tissue.

7. Remove serum by careful aspiration. Do not wash any further.

8. Incubation with primary antibody: Apply primary antibody (dilution according to manufacturer's recommendation). Incubate for 1-16 hours depending on antibody (ab) in humid chamber. Incubation time varies from antibody to antibody.

Specific binding of the antibody may be enhanced by prolonged exposure. On the other hand, non-specific binding may be minimal at short incubation times.

Important: include positive and negative control

9. Remove antibody solution by aspiration and rinse 1x in PBS, then wash for 1x5 minutes in large cuvette with PBS pH 7.2

10. Incubation with peroxidase- conjugated secondary antibody:

Prepare the following solution:

Peroxidase-conjugated secondary antibody:	according to own titration or manufacturer's suggestion.
Normal Goat Serum:	10%
(Normal Serum of tissue species; only necessary if the secondary antibody serum is not absorbed)	5%
PBS	Add to 100%

Incubate with 100-500µl per section in humid chamber for 60 minutes.

This step detects and enhances the presence of primary antibody.

11. Aspirate antibody solution and rinse 1x in PBS. Wash in PBS for 1x5 minutes in large cuvette (PBS pH 7.2)

12. Incubation with AEC substrate: Incubate with filtered AEC substrate solution (see above) for 12 minutes in cuvette at room temperature in the dark. Check color reaction.

13. Dip slide 3x in PBS, pH 7.2.

Color reaction is terminated as soon as the pH is adjusted to neutral.

14. Hemalum nuclear staining: Filter solution before use. Incubate slide for 1-2 minutes at room temperature, then carefully for 10 minutes under running cold tap water; apply one final rinse in distilled water.

15. Cover sections with Royalmount, 1-3 drops per section. Tissue must still be humid.

16. Polymerization: Put slide on 60°C heating plate for at least 30 minutes.

17. Mount cover slip: Dip slide in xylene (hood), add 1-3 drops of DPX Mountant, dip in xylene again, slip slide on tissue and let dry.