IHC PROTOCOL - IMMUNOFLUORESCENCE DETECTION (IHC-IF)

Protocol for immunohistochemistry with fluorescent detection optimized for Triple A Polyclonals™ and PrecisA Monoclonals™ from Atlas Antibodies.

MATERIALS NEEDED

Cryosections mounted on SuperFrost slides
• Target retrieval solutions pH6 or pH9 (e.g. DAKO or ThermoFisher)
• Incubation chamber with wet Wettex stripes
• DAKO Cytomation pen
• 1xPBS
• Fluorophore-conjugated secondary antibodies for regular immunofluorescence
• Mounting media (e.g. ProLong Gold with or without DAPI)
• Coverslips
• Eppendorf tubes for dilution of antibodies
• Pipettes and pipette-tips.

CRYO SECTIONS

- **Perfusion-fixed,** 10-30% sucrose cryoprotected sections cut in a microtome at 14 µm, thaw-mounted on Super Frost slides. Dry sections on slides for additional 2 h at room temperature (RT). Rehydrate in 1xPBS 2x15 min, or proceed with antigen retrieval.

- **Snap-frozen** sections cut in a microtome at 14 µm thaw-mounted on Super-Frost slides and dried for 2h. Pre-fix sections in ice-cold paraformaldehyde (4% PFA) for 20 min (put frozen sections directly into 4% PFA, do not allow thawing). Rinse in 1x PBS 15 min.
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ANTIGEN RETRIEVAL FOR CRYO SECTIONS (OPTIONAL)

If target requires antigen retrieval, standard target retrieval (HIER) solutions can be used, but the temperature and time should be reduced.

HEAT-INDUCED EPITOPE RETRIEVAL

1. DAKO or Thermo HIER solution pH 6, a modified citrate buffer, 60-70ºC, 10 min.
2. DAKO or Thermo HIER solution pH 9, a Tris/EDTA buffer, 60-70ºC, 10 min.

NOTE: *The specified working dilutions of the primary antibodies are to be considered as a guideline only. Optimal dilutions must be determined by the user.*

IHC PROCEDURE

DAY I

- Draw a circle around each section with DAKO Cytomation pen.
- Rinse slides in 1x PBS for 15 min
- Block sections in 2% normal serum of the secondary antibody host, in PBS 30 min at RT (optional)
- Add primary antibodies (diluted in 1x PBS, containing
- 0.3% Triton, 0.01% NaAzide, 0.02% Bacitracin), approx. 180µl/slide.
- Incubate in a humidified chamber overnight at 4ºC.

DAY II

- Rinse slides in 1x PBS for 15 min at RT (under a black cover)
- Incubate with secondary antibody conjugated with fluorophore (1:80 -1:400 in PBS), 30 min at 37ºC or 1-2 h at RT.
- Wash in PBS 2x15 min at RT under a black cover
- Mount in anti-fading solution (e.g ProLong Gold)
- Leave slides for 24h at 4C before microscopy/scanning
- Store at 4C

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