ATLAS ANTIBODIES

IHC PROTOCOL - IMMUNOFLUORESCENCE DETECTION

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 2 h. 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.

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), let cure for 24 h at 4ºC
- Ready for microscopy/scanning
- Store at 4ºC.

Revision A, Oct 2017

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