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NB100-740 Protocol

Immunocytochemistry/Immunofluorescence Protocol for IL-22RA1 Antibody (NB100-740)

Immunocytochemistry/Immunofluorescence Protocol for IL-22RA1 Antibody (NB100-740):
https://www.novusbio.com/products/il-22r-alpha-1-antibody_nb100-740
Immunocytochemistry on frozen tissues section:

Fix the cells cytospun on slides or frozen tissue section in cold acetone (acetone prechilled to -20C) for 2-5 minutes.

Let the slides dry and then block endogenous peroxide activity by treating the slides with peroxide blocker (3% hydrogen peroxide) for 10 minutes.

If the secondary antibody is from rabbit then cells should be incubated with 2% normal rabbit serum in PBS for 30min.

Wash the slide in PBS x 5 then incubate with the antibody at appropriate dilution in PBS containing Ig free 2% BSA or 2% milk for 30-45 minutes at room temperature.

Wash 5 times with PBS and then incubate with secondary HRP-conjugated antibody at a dilution optimized previously for 30 min-1hr at room temperature.

(If using primary cells from lymphoid organs containing B cells as well the secondary antibody must be specifies specific to minimize staining of B cells by secondary antibody alone).

Wash 5 times with PBS and develop the stain by adding the DAB or AEC and counter stain with hematoxylene.

Immunohistochemistry on Paraffin sections

Paraffin sections of tissues fixed in 10% formalin were used.

Preparation of slides: Place slides in 60 C oven overnight after cutting. Then follow the procedure below:

1) xylene 2x10min
2) 100% alcohol 2x10min
3) 95% alcohol 5 min
4) 80% alcohol 5 min
5) rinse in distilled water 2x2 min
6) antigen retrieval if necessary: Sections were treated with antigen retrieval solution (pH 6.0, pH 8.0) and steamed for 20 minutes followed by cooling for 20 minutes.
Block the endogenous peroxide by incubating the sections with 3% H2O2 for 15 minutes at room temperature (RT).

Wash the sections with PBS and incubate with 2% normal rabbit serum at room temperature to block the non-specific binding sites.

Wash the section with PBS and incubate with primary antibody at recommended dilution for 60 minutes at RT.

Wash the slides and incubate at RT with biotinylated rabbit anti-goat IgG.

A key step is blocking with normal rabbit sera prior to incubation with the secondary antibody.