ABGENT
primary antibody company

ABGENT CUSTOM SERVICES:

Immunohistochemistry Protocol

Preparing Tissue Sections for Immunostaining:

- Fix the tissue in 10% formalin at 4 ℃ overnight.
- Embed fixed tissue in paraffin.
- Mount tissue sections on slides.
- Clear the paraffin with xylene for ten minutes; move slides to a fresh dish of xylene for an additional ten minutes. **NOTE:** Perform all xylene washes in a fume hood!
- Rinse the slides twice for 2 minutes in 100% alcohols (18:1:1 100% ethanol: 100% methanol: 100% isopropanol).
- Rinse the slides twice for 2 minutes in a 95% solution of the 100% alcohols.
- Place slides in an 80% solution of the 100% alcohols for 2 minutes, followed by deionized water for 5 minutes.
- Rinse slides several times with fresh deionized water followed by another five minutes wash using fresh water.

Sodium Citrate Antigen Retrieval:

- Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 totals) to ensure even heating.
- Place rack in 600 ml of 10 mM sodium citrate, pH 6.0 in a glass 2 L-beaker. Mark a line at the top of the liquid on the beaker.
- Microwave for 20 min total, replacing evaporated water every 5 min.
- Cool slides for 20 min.
- Wash 4 × 3 min in ddH_2O, and 3 min in 1 × PBS.

Blocking

- Block endogenous peroxidases by soaking slides in a solution of 90% methanol/3% H_2O_2 for 15 minutes at room temperature. Wash 3 × in PBS.
- Immerse slides in a dish containing blocking buffer (serum from host species of secondary antibody to be used, diluted 1:10 in TBS). Incubate at 37 ℃ for one hour.

Incubation with Primary Antibodies

- Cover the tissue sections with primary antibody diluted in blocking buffer. Antibody is diluted 1:50 and 1:100. Incubate for 1 hour at 37 ℃.
- Blot excess liquid from slides and rinse three times in PBS for five minutes each wash.

Incubation with Secondary Antibodies

- Cover the tissue sections with secondary antibody diluted in blocking buffer according to manufacturer's instructions. We routinely use prediluted universal secondary antibody (Jackson ImmunoResearch Laboratories). Incubate at 37 ℃ for

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30 min.
- Blot excess liquid and rinse twice in TBS for five minutes each wash.

Counterstaining and Visualization

- Counterstain with Hematoxylin.
- Rinse several times in deionized water. Blot excess water around tissue, then apply one drop of mounting media to tissue and place coverslip over slide. Seal with nail polish.

Citrate Solutions:

Description: Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, thereby giving weak or false negative staining for immunohistochemical detection of certain proteins. The citrate based solution is designed to break the protein cross-links, thereby unmasking the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections and enhancing the staining intensity of antibodies.

Sodium Citrate Buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0):

Tri-sodium citrate (dihydrate) --------- 2.94 g
Distilled water ---------- 1000 ml
Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 ℃ for longer storage.

Citrate Buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0):

Citric acid (anhydrous) ---------- 1.92 g
Distilled water ---------- 1000 ml
Mix to dissolve. Adjust pH to 6.0 with 1N NaOH and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 ℃ for longer storage.

Washing Buffer:

1 × PBS:

NaCl ---------- 8 g
KCl ---------- 0.2 g
Na_2HPO_4---------- 1.44 g
KH_2PO_4---------- 0.24 g
Distilled Water---------- 800 mL
Adjust pH to 7.2 with HCl.
Adjust volume to 1 L with additional H_2O

Normal Serum Blocking Buffer:

2% serum from host species of secondary antibody (blocking)
1% BSA (stabilizer)
0.1% cold fish skin gelatin (blocking)
0.1% Triton X-100 (penetration enhancer)
0.05% Tween 20 (detergent and surface tension reducer)
0.05% sodium azide (preservative)
Dissolve in 1 × PBS
Mix well and store at 4 ℃.

Avidin/Biotin Block:

Avidin 0.001% in 1 × PBS
Biotin 0.001% in 1 × PBS
Store these blocking solution at 4 ℃.

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Primary Antibody Dilution Buffer:

1%BSA (stabilizer and blocking)
0.1% cold fish skin gelatin (blocking)
0.05% sodium azide (preservative)
0.01M PBS pH7.2
Note: 1) Antibodies diluted using this buffer can be stored at 4℃ for 6 months without reducing binding activity. 2) This buffer cannot be used for diluting HRP conjugated antibodies since sodium azide is an inhibitor of HRP.

Peroxidase Blocking Solution (3% H_2O_2 in PBS):

30% H_2O_2 ---------- 2 ml
1 × PBS ---------- 18 ml
Mix well and store at 4℃ for up to 3 months.
This solution is recommended for paraffin sections

References:

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