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RABBIT ANTI-TAURINE POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5022

LOT NUMBER:

QUANTITY: 2000 Tests (500 μL)

SPECIFICITY: Taurine. The antibody has been calibrated against a spectrum of antigens to assure hapten selectivity and proper affinity. No measurable glutaraldehyde-fixed tissue cross-reactivity (<1:1000) against L-alanine, γ-aminobutyrate, 1-amino-4-guanidobutane (AGB), D/L-arganine, D/L-aspartate, L-citrulline, L-cysteine, D/L-glutamate, D/L-glutamine, glutathione, glycine, L-lysine, L-ornithine, L-serine, L-threonine, L-tryptophan, L-tyrosine.

IMMUNOGEN: Taurine-glutaraldehyde

APPLICATIONS: Immunohistochemistry using silver-intensified immunogold or fluorescence (see recommended protocol). Samples should be fixed with 0.05% - 0.25% glutaraldehyde for optimum detection.

*****This antibody has also been used and found to work with a zero-low glutaraldehyde / high paraformaldehyde fixation (4% paraformaldehyde in 0.1M phosphate buffer / 3% sucrose fixative). The minimum glutaraldehyde concentration for AB5022 is 0.00%. See protocol that follows. Performance is good with frozen sections, Vibratome sections and tissue culture formats, when penetrating reagents such as 0.3% Triton X-100 are used.

Optimal working dilutions must be determined by the end user.

DILUTION: Prepare enough of the AB5022 for your days use by diluting 100X with 1% GSPBT.

FORMAT: Purified immunoglobulin.

PRESENTATION: Liquid in stabilizing buffer (Phosphate buffer with sterile goat serum) containing 0.05% thimerosal.

STORAGE: Maintain stock at 2-8°C in undiluted aliquots for up to 6 months. This stock is extremely stable under normal use and routine storage at 2-8°C. **Do not freeze this stock**.

RECOMMENDED PROTOCOL for AB5022

CONDITIONS

All procedures may be carried out at room temperature. Though the exact dilutions for all applications can not be predicated, it is unlikely that deviations from the calibrated concentration will be needed. The 100X dilution is calibrated to optimize the detection of antigen levels over a 2 log unit range. Greater or lesser dilutions may be employed if unusually high (>20 mM) or low (<0.2 mM) intracellular hapten levels are anticipated or novel specimen conditions exist, such as low glutaraldehyde levels or unusual embedding protocols. Greater dilutions will likely result in decreased maximum signal strength whereas more concentrated solutions will compress the dynamic range of detection.

TECHNICAL NOTES

The AB5022 is optimized for use with immunogold or fluorescence detection. Visualization with enzyme-linked methods can be used but they typically compress the dynamic range of detection. Use of high gain methods such as tyramides will require the user to perform independent calibrations. Use with frozen sections is possible but will not yield optimal images as IgGs will penetrate aldehyde cross-linked tissue poorly and most amino acids are present at such high levels that prozone effect occurs. Use in whole mount or vibratome sections is not recommended for similar reasons.

A. BASIC WORKING REAGENTS

Antibody Working Solution: Dilute stock 100X with 1% GSPBT.

Sodium Ethoxide (see below)
Anhydrous ethanol/methanol
Sodium metaperiodate
PB = 0.1 M phosphate buffer, pH 7.4
PBT = PB + 0.05% thimerosal, pH 7.4
1% GSPBT = 1% goat serum in PBT
Second Antibody = 1 nm Gold conjugated or fluorophore conjugated anti-rabbit IgG
Mounting medium

Silver Intensification Solutions:
Stock A = 0.2 M citrate buffer pH 4.85 (critical pH! - 4.9 or higher is not acceptable - store in air-free vessel)
Stock B = 0.5 g hydroquinone in 15 mL deionized water (mix up fresh every day)
Stock C = 1% aqueous silver nitrate (may be stored indefinitely at room temp. wrapped in foil)
Working solution = 5 mL Stock A + 1 mL Stock B + 1 mL Stock C, in that order
Stop solution = 5% acetic acid

Making your own sodium ethoxide stocks:

Place approximately 1 inch of sodium hydroxide pellets in a 500-1000 mL heavy glass storage bottle with a frosted glass stopper covered in wax film. Cover the pellets with at least 3 inches of ethanol and place stoppered in a hood. Shake daily until the solution becomes dark brown and syrupy (7-14 days), whence it is ready to use. Add replacement ethanol as the stock is used. It is very important that absolutely no water come in contact with either the stock or the working solution as inclusion of water will result in etching of protein in addition to plastic. Use glass or metal slide trays. **Mark as a hazardous reagent. It is extremely caustic!**

RECOMMENDED PROTOCOL for AB5022

B. PROCEDURE

1. Sections of epoxy resin embedded tissue (from 100-1000 nm thick) on spot slides.
2. Deplasticize in 1:5 v/v solution of mature sodium ethoxide in anhydrous ethanol, 1.5 minutes/100 nm section thickness. Keep completely anhydrous at all times.
3. Wash in three 2 minute changes in anhydrous ethanol or methanol, followed by one 5 minute running tap water rinse and a dip in deionized water.
4. Dry with a dust-free air canister.
5. Osmicated specimens must be treated with fresh 1% sodium metaperiodate for 10 minutes followed by a 1 minute wash in PB. Dip in deionized water and dry. Otherwise, skip to Step 6.
6. Incubate with AB5022 for 4 hours to overnight at 25 µL/well. Cover with an inverted glass staining dish sealed with plastic wrap to prevent evaporation.
7. Flick off antiserum. Dip once in 0.1M PB to rinse off excess and wash 10 minutes in 1% GSPBT; use plastic mailing cassettes that hold 5 slides (requires about 15 mL of solution).
8. Dip slides in deionized water and dry with air canister. Incubate 60 minutes with 25 µL/well of Second Antibody diluted in 1% GSPBT (e.g. a 1 nm gold goat anti-rabbit IgG or a fluorophore conjugated anti-rabbit IgG). Silver-intensification methods are the most sensitive and are archival.
9. Flick off Second Antibody. Dip in 0.1M PB to rinse off excess. Wash in PB 1 hour in plastic cassettes.
10. Dip in deioized water and dry with an air canister. Mount in medium of your choice if using fluorescence. Otherwise, go to step 10.
11. Prepare fresh silver intensification solutions. Stock Solutions A & C may be prepared in advance. Prepare Solution B fresh and use within 1 hour.
12. Use the working solution immediately as it lasts only 10 minutes. Expose sections to the solution for 6-8 minutes in a dark location, such as under an aluminum cover. You needn't work in absolute darkness, but the background decreases slightly if the intensification occurs away from bright light.
13. Stop the reaction with a brief dip in 5% acetic acid.
14. Wash for 10 minutes in deionized water and dry with an air canister.
15. Cover slip in a medium of your choice.

PROTOCOL for ZERO-LOW GLUTARALDEHYDE / HIGH PARAFORMALDEHYDE FIXATION for AB5022

A. BASIC WORKING REAGENTS

Antibody Working Solution: Dilute stock 100X with 1% GSPBT.

Sodium Ethoxide (see below)
Anhydrous ethanol/methanol
PB = 0.1 M phosphate buffer, pH 7.4
PBTX = PB + 0.05% thimerosal, pH 7.4 + 0.3% Triton X-100.
1% GSPBTX = 1% goat serum in PBTX
Second Antibody = Biotinylated anti-rabbit IgG
Detection reagent = 1 nM Gold conjugated or fluorophore (e.g. Cy3™) conjugated streptavidin
Mounting medium

PROTOCOL for ZERO-LOW GLUTARALDEHYDE / HIGH PARAFORMALDEHYDE FIXATION for AB5022

Silver Intensification Solutions:
Stock A = 0.2 M citrate buffer pH 4.85 (critical pH! - 4.9 or higher is not acceptable - store in air-free vessel)
Stock B = 0.5 g hydroquinone in 15 mL deionized water (mix up fresh every day)
Stock C = 1% aqueous silver nitrate (may be stored indefinitely at room temp. wrapped in foil)
Working solution = 5 mL Stock A + 1 mL Stock B + 1 mL Stock C, in that order
Stop solution = 5% acetic acid

B. PROCEDURE

Follow the standard procedure above with the following substitutions/insertions.

Step 4: Dry with a dust-free air canister. Block 60 minutes with 1% GSPBTX at 25 µL per well. Flick off droplets and do not rinse. Probe immediately.

Step 8: Dip slides in deionized water and dry with air canister. Incubate 60 minutes with 25 µL/well of biotinylated second antibody diluted in 1% GSPBTX.

Step 9: Flick off Second Antibody. Dip in 0.1M PB to rinse off excess. Wash in GSPBTX 1 hour in plastic cassettes.

Step 9a: Dip slides in deionized water and dry with an air canister. Incubate 60 min with 25 µL/well of 1 nm Gold conjugated or fluorophore (e.g. Cy3) conjugated strepavidin.

Step 9b: Flick off droplets. Dip in 0.1M PB to rinse off excess. Wash in GSPBTX 1 hour in plastic cassettes.

Disclaimer: As conditions of use are outside our control, Millipore makes no warranties, express or implied, and assumes no liability in connection with the use of this protocol.

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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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