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Polyclonal Antibody to Glutamate

Catalog No.: BP139

Quantity: 1 ml

Concentration: 0.17 mg/ml (OD280, lot specific)

Host: Rabbit

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Immunogen: Glutamate-hemocyanine conjugate

Format: This antibody is supplied as liquid Ig fraction, purified by Ammonium Sulphate fractionation, in PBS pH 7.2 containing 1% BSA and 0.09% Sodium Azide as preservative.

Applications: Immunohistochemistry (1/10-1/5,000). Has been used in a procedure based on that described in (1). Has been used at various dilutions with different detection methods on both rat and cat sections. Othwer applications not tested. Optimal dilutions are dependent on conditions and must be determined by the user.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody is reactive with various glutamate containing di-peptides (Glu-Glu, Asn-Glu etc.). Absorption studies indicate no cross reactivity with aspartate, GABA, beta-alanine, glycine, asparagine, leucine, isoleucine and glutamine. Reacts with rat and cat.

Storage: Store at -20d?°C in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.

General Readings:

1. Techniques in IHC, Vol 4, London: Academic Press, 253-272.
J. Histochem Cytochem 29, 1397-1404, 1981.
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Exp Brain Res 93: 95-103, 1993.
Psychopharmacology 125: 238-247, 1996.
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Synapse 18: 205-217, 1994.
Brain Res 648 181-195, 1994.
J. Comp Neurol 336, 321-330, 1993.
J. Comp Neurol 322, 519-527, 1992.
Brain Research 627: 89-103, 1993.
Brain Res 635: 187-195, 1995.
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Recommended Procedure for Immunocytochemistry

Animals should be anaesthetized with phenobarbital or chloral hydrate and perfused with fixatives intracardially at room temperature.

For light microscopy (vibratome or paraffin): Use 4% CD (carbodiimide; cyanimide) in 0.1 M phosphate buffer followed by 4% paraformaldehyde (PF) in same buffer. Alternatively, a saline rinse may be followed by perfusion with 4% PF and 0.5% glutaraldehyde (GA).

The inclusion of 0.2% picric acid in the perfusion mixture is optional in either procedure.

The concentration of GA may be adjusted according to individual requirements. For example, if ultra-structural preservation is important, higher concentrations of GA are recommended. When amino acid immunocytochemistry is combined with peptide localisation, lower concentrations of GA are recommended.

For pre-embedding electron microscopy staining: Rinse with PBS, followed by a mixture of 2% CD and 0.2% - 1% GA in phosphate buffer. For best results, leave animal undisturbed on the operating table for approximately 30 minutes, then perfuse with a second fixative (4% PF).

After perfusion, brain and/or spinal cord should be removed carefully and postfixed in a 4% PF solution in PBS for 12-72 hours at 4ºC. This step appears to be essential for good results. The optimal period of postfixation should be determined individually.

Sectioning: for free-floating sections for light microscopy or for pre-embedding EM staining, section by vibratome, 50 mm in iced saline (Ultracryostat may be used for thin section). Collect vibratome sections in PBS.

Immunocytochemical Staining: All steps are to be done at room temperature, except incubation with the primary antibody for paraffin sections. For free-floating sections, all incubations are on a rotary shaker at 100 rpm.

Please note in most cases a highly sensitive detection method has been used with this antibody e.g. double PAP and avidin-biotin