ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Dopamine Antiserum

Cat # DOP11-S Rabbit Anti-Dopamine Antiserum **SIZE**: 100 ul.

Source of Antigen and Antibodies

Dopamine was coupled to BSA using glutaraldehyde procedure and polyclonal antibodies produced in rabbits.

Antigen	**Dopamine** was coupled to BSA
Ab Host/type	Rabbit, Polyclonal antiserum # DOP11-S
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)

100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage

The product may be used to localize dopaminergic neurons of substantia nigra and the A10 regions as well as dopaminergic nerve terminals of locus coeruleus. Antibody dilution will depend upon the experimental conditions and may vary from 1:250-1:1000. Antibody tier by dot blot is 1:5K using dopamine coupled to a carrier protein.

Since the antibodies are made to the BSA-conjugates, the use of BSA in all buffers and antibody diluents must be avoided.

Specificity & Cross-reactivity

Anti-dopamine should react with dopamine from all species. Antibody labels dopaminergic neurons of substantia nigra and of the A10 region as well as dompaminergic nerve terminals of locus coeruleus. Noradrenaline-BSA conjugate does not inhibit DOP11-S antibody activity.

General References:

(1) Yang C et al (1991) J. Comp. Neurol. 312, 19-32
(2) McRae-Degueurce, A (1986). *Brain Research* **376**:217

Suggested Protocol for use with Rabbit anti-Dopamine (refs 2)

Prepare andu use fresh solutions.
Pre-wash (PS) Solution: Sodium meta-bisulfite (MBS 1%) and Cacodylate (50mM). In these proportions the pH should be 6.2-6.5 without any need of adjustment.

Fixation solution (FS): Mix Glutaraldehyde (5%) into a solution of cacodylate (100 mM) and MBS (1%). The pH should be between 7.0-7.5. Variations in the pH of this solution have been attributed to the oxidation of MBS which of course would need to be replaced.

Perfusion & Post Fixation:

a) A perfusion pump is recommended. First 30 mL of PS is transfused intracardially, then immediately followed by 500 mL of FS at 200 mL/minute.
b) Post fix: 15 minutes in FS.
c) Post fix washing: the brains are extensively washed in 1% MBS in 50mM Tris adjusted to pH 7.5 using concentrated NaOH (Tris-MBS).

Tissue Sectioning:

Vibratome sectioning: the sections should be floated in Tris-MBS.
Cryostat sectioning: dissected brain tissue is rinsed overnight in Tris-MBS containing 10% sucrose. The tissue is frozen the following day using compressed CO2 gas. After cutting, the frozen sections are mounted on gelatin-coated slides. When the sections are dry, the slides are immersed into Tris-MBS and maintained so until use. 30 minutes prior to addition of the primary antibody, normal goat serum is added to a concentration of 2%.

Primary Antibody:

Antiserum should be used at dilutions of 1:250-1:1,000 using Tris-MBS containing 0.5% Trition and 1% normal goat serum. The antibody should be incubated 15 hours at +2-8℃ or room temperature. Optimal working dilutions must be determined by end user.

Post Treatment:

The immunocytochemistry procedure is then continued with the PAP method or the avidin-biotin peroxidase system. Note: since MBS is known to inhibit PAP or avidin-biotin peroxidase, it should be omitted. 40 mM Tris-HCl with 9% NaCl adjusted to pH 7.6 (using concentrated HCl) should be used.
Notes: MBS is essential in the pre-wash and the glutaraldehyde solution since this antioxidant protects the dopamine molecule from being released.

*This product is for In vitro research use only.

Related material available from ADI

Anti-Dopamine receptors D1-D5, DBH, DDC
Anti-Rabbit IgG-HRP Conjugate and ECL Reagents
NuGlo Chemiluminescent Substrate Kit
Western Blot Recycling Kit (Strips blots in 5 minutes)

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