



ANTI-CONJUGATED D.AMPHETAMINE ANTIBODIES

CATALOG NUMBER : AP166

TARGET : Conjugated D.Amphetamine

IMMUNOGEN : Synthetic D.Amphetamine conjugated to protein carriers.

SPECIFICITY : Using a conjugate D.Amphetamine-protein carrier, antibodies specificity was performed with an ELISA test by competition experiments with the following compounds :

<i>Compounds</i>	<i>Cross-reactivity ratio (a)</i>
D.Amphetamine-G-BSA	1
L.Phényl-alanine-G-BSA	1/>50,000
L.Tyrosine-G-BSA	1/>50,000
Tyramine-G-BSA	1/>50,000
Octopamine-G-BSA	1/>50,000

(a) : D.Amphetamine-G-BSA concentration/ other conjugated competitors concentration at half displacement.

BSA = Bovine Serum Albumin

G = Glutaraldehyde

RAISED IN : Rabbit

CLONALITY : Polyclonal

ISOTYPE : IgG

PURITY : Antiserum previously absorbed on protein carriers, and purified.

FORM : Lyophilized

RESEARCH AREAS : drugs, neurobiological mechanisms

STORAGE INSTRUCTIONS : Lyophilized vial must be stored at 4°C in a dry area. After reconstitution with 50µl of distilled water and 50µl of glycerol, the aliquot can be stored at -20°C, and is stable at least 2 years.

CORRESPONDING ANTIGEN: Gemacbio sells the corresponding antigen : D.Amphetamine(BSA) conjugate (catalog number: AG166).



EXAMPLE OF MATERIAL AND METHODS

• Example of immunohistochemistry protocol

Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg) :

1-The animals can be deeply anaesthetized for example with urethane (0.5-1.5g/kg, intraperitoneal).

2-Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:

- a) 300 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (two minutes).
- b) 600 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (ten minutes).
- c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
- d) Before the organs will be cut on a freezing microtome, we must include the organs in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the organs sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

Around 50 µm-thick serial sections will be obtained, kept at 4° C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of immunohistochemical protocol

1-In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH₃ (20%), H₂O₂ (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H₂O₂ and 66% of methanol).

2-Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)

3-Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).

4-Incubate at room temperature (1h 30min) and overnight at 4° C in the same mixed solution containing anti-conjugated D. Amphetamine antibodies (diluted 1/1,000 to 1/5,000; as recommended dilution).

5-Then, the sections will be wash in PBS (30 min).

6-After that we will incubate for 60 min at room temperature with biotinylated anti-rabbit immunoglobulin (Vector) diluted 1/200 in PBS.

7-Wash during 30 min with PBS.

8-Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).

9-After that we will wash the sections in PBS (30 min)

10-Wash with Tris-HCl buffer (pH 7.6)(10 min).

11-The tissue-bound peroxidase will be developed with H₂O₂ using 3, 3' diaminobenzidine as chromogen.

12-Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).