



ANTI-CONJUGATED 3-HYDROXYKYNURENINE ANTIBODIES

CATALOG NUMBER : AP164

TARGET : Conjugated 3-Hydroxykynurenine

IMMUNOGEN : Synthetic 3-Hydroxykynurenine conjugated to protein carriers.

SPECIFICITY : Using a conjugate 3-Hydroxykynurenine-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>
3Hydroxykynurenine-BSA	1
L.Kynurenine-BSA	1/500
3-Hydroxyanthranilic acid-BSA	1/1,000
L.Tryptophan-BSA	1/1,000

(a) : 3Hydroxykynurenine-BSA concentration/ other conjugated competitors concentration at half displacement.
BSA = Bovine Serum Albumin

RAISED IN : Rabbit

CLONALITY : Polyclonal

ISOTYPE : IgG

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

FORM : Lyophilized

RESEARCH AREAS : Neuroscience, Neurobiology, Alzheimer disease

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 : 3-Hydroxykynurenine(BSA) conjugate (catalog number: AG164).

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 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1} M, pH 5.4, and with the following solutions:
 - a) 200 ml of a solution containing MES 10^{-1} M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1} M (two minutes).
 - b) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
 - c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
 - c) Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of immunohistochemical protocol

- 1-In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 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 3-Hydroxykynurenine 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 H_2O_2 using 3, 3' diaminobenzidine as chromogen.
- 12-Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).