



ANTI-CONJUGATED NICOTINAMIDE ANTIBODIES

CATALOG NUMBER : AP121

TARGET : Conjugated Nicotinamide (Vitamin PP)

IMMUNOGEN : Synthetic Nicotinamide conjugated to bovine serum albumin (BSA)

SPECIFICITY : Using a conjugate Nicotinamide-protein carrier (BSA), antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

<i>Compounds</i>	<i>Cross-reactivity ratio (a)</i>
Nicotinamide	1
Pyridoxine-BSA	1/30
Pyridoxal-BSA	1/9,000
Pyridoxal (reduced)-BSA	1/10,000

(a) : Nicotinamide-BSA concentration/conjugated compound concentration at half displacement.

RAISED IN : Rat

CLONALITY : Polyclonal

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

FORM : Lyophilized

TESTED APPLICATION : Immunocytochemistry

RESEARCH AREAS : Biochemistry (metabolic ways).

NOTES : Principal name Nicotinamide or Amide nicotinic

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 : Nicotinamide (BSA) conjugate (code number : AG121)

REFERENCE

• MANGAS A, COVENAS R, BODET D, DULEU S, MARCOS P, GEFFARD M Vitamins in the monkey brain : an immunocytochemical study. J. Chem. Neuroanat. (2009) 38(1), 1-8.

EXAMPLES OF MATERIAL AND METHODS

• Example of Immunohistochemistry application

Perfusion protocol for Adult male monkeys (*Macaca fascicularis*)(weight 3-3.5 kg) :

1-The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).

2-Heparinized, and perfused via the ascending aorta with 300 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:

- a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
- b) 2,500 ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
- c) 5,000 ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
- d) 2,000 ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
- e) Dissect out the brains and place in 10% glycerol and 2% dimethylsulfoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

Around 50 µm-thick serial sections will be obtained, kept at 4° C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, 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 Nicotinamide antibodies (diluted 1/500–1/1,000; as recommended dilutions).

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-rat 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).