

**ANTI-CONJUGATED L.KYNURENINE ANTIBODY**

**CATALOG NUMBER : AM009**

**DESCRIPTION :** Monoclonal antibody was obtained after BALB/c mouse immunisation with the conjugate : L.Kynurenine-Protein Carriers (PC) and hybridization of spleen cells with the myeloma cell line SP2/O/Ag14. Ascite production was performed in BALB/c mice.

**TARGET :** Conjugated L.Kynurenine

**IMMUNOGEN :** Synthetic L.Kynurenine-PC

**SPECIFICITY :** Using a conjugate L.Kynurenine-PC, antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

<i>Compounds</i>	<i>Cross-reactivity ratio<sup>(a)</sup></i>
L.Kynurenine-BSA	1
D.Kynurenine-BSA	1/7
Picolinic acid-BSA	1/3,000
Xanthurenic acid-BSA	1/>50,000
Quinaldic acid-BSA	1/>50,000
L.Tryptophan-BSA	1/>50,000
3OH Kynurenine-BSA	1/>50,000
Anthranilic acid-BSA	1/>50,000
Quinolinic acid-BSA	1/>50,000
Kynurenic acid-BSA	1/>50,000
Free L.Kynurenine	1/>50,000

(a) : L.Kynurenine-PC concentration/Other conjugated close related compounds concentration at half displacement.  
PC : Protein Carrier.

**RAISED IN :** Mouse

**CLONALITY :** Monoclonal

**CLONE NUMBER:** KYNU4A6

**ISOTYPE :** IgG 1, Kappa.

**PURITY :** IgG 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: L.Kynurenine(BSA) conjugate (catalog number: AG147)



## **EXAMPLES 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) 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).
- d) 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.
- e) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains 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  $\mu$ m-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  $\text{NH}_3$  (20%),  $\text{H}_2\text{O}_2$  (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of  $\text{H}_2\text{O}_2$  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 L.Kynurenine antibody (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-mouse 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  $\text{H}_2\text{O}_2$  using 3, 3' diaminobenzidine as chromogen.

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