PRODUCT INFORMATION

ANTI-CONJUGATED D-GLUTAMATE (D-GLUTAMIC ACID) ANTIBODIES

Ref: AP030

TARGET: Conjugate D-Glutamic Acid

IMMUNOGEN: Synthetic D-Glutamic Acid conjugated to protein carrier (PC)

SPECIFICITY: Using a conjugate D-Glutamic Acid-PC, antibody specificity was performed with an ELISA by competition experiments with the following compounds:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cross-reactivity ratio (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glutamate-PC</td>
<td>1</td>
</tr>
<tr>
<td>L-Glutamate-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Glutamine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>L-Glutamine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>L-Aspartate-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Aspartate-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>GABA-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>Taurine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Cystein-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Methionine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Tryptophan-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>D-Tyrosine-PC</td>
<td>1/&gt;50,000</td>
</tr>
</tbody>
</table>

(a): Glutamate-PC concentration/other conjugated amino acid concentration at half displacement
RAISED IN: Rabbit

CLONALITY: Polyclonal

ISOTYPE: IgG

PURITY: Antiserum preabsorbed on protein carriers, purified by ammonium sulfate and/or by high trap column

FORM: Lyophilized

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.

RESEARCH AREAS: Neurobiology, Neurodegenerative diseases, Biochemistry, Pharmacology

TESTED APPLICATION: Immunocytochemistry. Optimal dilutions should be determined by each laboratory for each application.

CORRESPONDING ANTIGEN:
Gemac sell the corresponding antigen: D-Glutamate conjugate (ref: AG030)

REFERENCE
EXAMPLE OF PROTOCOL

_Perfusion protocol for adult male Sprague-Dawley (weight around 0.5kg)_

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

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

   a) 300mL of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).

   b) 600mL of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M 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 4ºC for twelve to sixteen hours.

   d) 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 50µm-thick serial sections will be obtained, kept at 4º C in PBS (0.1M, 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 0.15M 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 (1h30min) and overnight at 4º C in the same mixed solution containing D-Glutamic acid antiserum (diluted 1/1,000-1/5,000; 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 1h 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).

Hereafter, an example of the immunoreactive cell bodies that have been found using this protocol.

*Immunoreactive cell bodies containing D-Glutamate in the ventrolateral region of the periaqueductal gray of the brain rat. Scale Bar: 50µm.*