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**Anti-Conjugated D-Glutamic Acid (D-Glutamate)
RABBIT POLYCLONAL**

Catalog Number: AB-T045

Example of ELISA protocol used to test conjugated D-Glutamate:

1. Coating of conjugated D-Glutamate ($10\mu\text{g/ml}$) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C .
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated D-Glutamate antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, $200\mu\text{l}$ by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. $200\mu\text{l}$ of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating $200\mu\text{l}$ by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of $50\mu\text{l}$ of 2M HCl.
9. The optical density will be measured at 492nm.

**Anti-Conjugated D-Glutamic Acid (D-Glutamate)
RABBIT POLYCLONAL**

Catalog Number: AB-T045

Example of Immunohistochemistry used to test conjugated D-Glutamate:

Perfusion Protocol for adult male Sprague-Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized 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 4°C 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 4°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 4°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-rat 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).