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**Anti-Conjugated D-Aspartic Acid (D-Aspartate)  
RABBIT POLYCLONAL**

**Catalog Number:** AB-T046

**Example of ELISA protocol used to test conjugated D-Aspartic acid:**

1. Coating of conjugated D-Aspartic acid ( $10\mu\text{g/ml}$ ) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer  $0.05\text{M}$  (pH 9.6), during sixteen hours at  $4^{\circ}\text{C}$ .
2. Saturation of well plates with a solution of PBS (pH 7.3) containing  $1\text{g/l}$  of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at  $37^{\circ}\text{C}$ ).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated D-Aspartic acid antibodies will be diluted ( $1/1,000$ - $1/5,000$ ) in PBS Tween containing  $1\text{g/l}$  BSA,  $1\text{g/l}$  of BSA-G and 10% of glycerol,  $200\mu\text{l}$  by well plate (incubating during 2 hours at  $37^{\circ}\text{C}$ ).
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  $1\text{g/l}$  of BSA, will be applied by well plate (during one hour at  $37^{\circ}\text{C}$ ).
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  $0.1\text{M}$ /phosphate  $0.2\text{M}$  (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  $2\text{M}$  HCl.
9. The optical density will be measured at  $492\text{nm}$ .



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**Anti-Conjugated D-Aspartic Acid (D-Aspartate)  
RABBIT POLYCLONAL**

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**Example of Immunohistochemistry used to test conjugated D-Aspartic acid:**

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

1. The animals can be deeply anesthetized for example 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 50 $\mu$ m-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 NH<sub>3</sub> (20%), H<sub>2</sub>O<sub>2</sub> (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H<sub>2</sub>O<sub>2</sub> 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 D-Aspartic acid antiserum (diluted 1/1,000-1/5,000; 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 H<sub>2</sub>O<sub>2</sub> using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).