ADVANCED TARGETING SYSTEMS

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Anti-Conjugated D-Aspartic Acid (D-Aspartate) RABBIT POLYCLONAL AB-T046

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

1. Coating of conjugated D-Aspartatic acid (10μg/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-Aspartatic acid 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, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl 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 200μl 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 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Example of Immunohistochemistry used to test conjugated D-Aspartatic 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 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 0.15 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 immunogammaglobulin (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).