ADVANCED TARGETING SYSTEMS

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Anti-Conjugated L-Aspartate
RABBIT POLYCLONAL
AB-T022

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

1. Coating of conjugated L-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 L-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.

Example of Immunohistochemistry used to test conjugated L-Aspartatic acid:

Detection of conjugated L-Aspartatic acid in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated L-Aspartatic acid antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Example of immunohistochemistry used to test conjugated L-Aspartate and Taurine:

Detection of conjugated Aspartate and Taurine in cockroach brain

1. Post fixation: For aspartate and taurine immunostaining, brains will be fixed overnight in 4% paraformaldehyde / 1% glutaraldehyde in 0.1M phosphate buffer (pH 7.3).
2. Tissue sectioning: Brains will be embedded in 7-8% agarose or gelatine/albumin and sectioned at 60-100 μm with a vibratome (Leica).
3. Application of anti-conjugated antiserum: After washing with phosphate buffer containing 0.3% TritonX100 (PBST), sections will be incubated in the same buffer with 10% normal swine serum (Dako Corp.).
   Application of anti-conjugated Aspartate antiserum or Taurine antiserum: Sections will be then incubated overnight with aspartate antiserum (1/1,000) or taurine antiserum (1/1,000) at room temperature.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

4. Revelation: Second antibody: After a PBST wash, secondary antibodies will be applied overnight. These consisted of goat anti-rabbit immunoglobulins conjugated to Texas Red (1/250, Jackson ImmunoResearch Laboratories) or anti-rabbit immunoglobulins conjugated to Alexa 568 (Molecular probes).
   After a final wash, sections will be mounted on slides and cover–slipped under 80% glycerol.

An immunofluorescence double staining method will be used to simultaneously reveal taurine and aspartate immunoreactivity in the gelatin sections. This entailed two sequences of primary and secondary labeling.

1. Aspartate antiserum (1/1,000) will be applied to gelatin sections overnight.
2. After a 6-hour wash, sections will be exposed for 12-18 hours to biotinylated swine anti-rabbit immunoglobulins diluted to 1/250 (Dako Corp.) in PBST.
3. Sections will be next washed for 6 hours in 0.01M PBST. This step will be followed by an overnight incubation in streptavidin-fluorescein (1/100, Jackson ImmunoResearch Laboratories). The above concentration of biotinylated swine anti-rabbit immunoglobulins blocked all antigen sites of the primary antibody.
4. After completing this stage, sections will be washed for 6-8 hours and then incubated overnight with rabbit taurine antiserum (1/500).
5. After a 6 hour wash in PBST, the sections will be incubated overnight with goat anti-rabbit immunoglobulins conjugated to Texas Red (1/250) or Cy5 (1/250). As a control, PBST replaced the aspartate antiserum and the taurine antiserum. No interaction between the reagents of the first and second layers of antibodies will be observed.