Advanced Targeting Systems

i-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
RABBIT POLYCLONAL
AB-T067

Example of ELISA protocol used to test conjugated L-dihydroxyphenylalanine:

1. Coating of conjugated L-dihydroxyphenylalanine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Preabsorbed L-dihydroxyphenylalanine antiserum will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a 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, to obtain the different values.

Example of Immunohistochemistry used to test conjugated L-dihydroxyphenylalanine:

Detection of conjugated L-DOPA 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. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated L-DOPA 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.

6. 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-dihydroxyphenylalanine:

Double detection of conjugated L-DOPA and Dopamine in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 5% glutaraldehyde (G), 0.9% sodium metabisulfite (SMB) solution in 0.1cacodylate buffer pH 7,4.
2. Post fixation: 2h, 4°C in the same fixative solution.
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated antiserum: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB). Then, the sections will be washed in the same solution (12h, 4°C) and incubated in Tris-SMB containing 3% non specific serum and 0.1% Triton X100 (8h at 4°C).
5. Application of anti-conjugated L-DOPA antibodies: Free floating adjacent sections will be incubated (24h, 4°C) with a polyclonal antiserum against conjugated L-DOPA (1/,1000 to 1/5,000), with a monoclonal antibody against conjugated DA (1/,1000 to 1/5,000), and with both. Antisera will be diluted in Tris-SMB, 1% non-specific serum, 0.2% Triton X100 solution.

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.

6. PAP procedure:
   Second antibody: After rinsing, sections will be incubated (12 hours at 4°C) with respectively swine anti-rabbit IgG antibodies (DAKO), goat anti-mouse IgG antibodies (DAKO) and both. Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum .
   PAP: Rinsed again, sections will be then incubated (1 hour at 37°C) with a 1/1,000 dilution of rabbit peroxidase anti-peroxidase (PAP) complex (DAKO) for single L-DOPA detection and 1/500 dilution of PAP mouse complex for DA detection.
   Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3'-diaminobenzidine (DAB ,Sigma) plus cobalt chloride (Sigma, 10mg/20ml) and 0.01% hydrogene peroxide (30vol., Merck).

   You must repeat the protocol: it needs that you must do step of PAP and develop with DAB after the second PAP with DADNi.

   For the double detection of L-DOPA and DA, the sections which have received anti-DA together with L-DOPA antibodies and then anti-rabbit together with anti-mouse secondary antibodies, will be incubated with PAP mouse complex, then revealed in DAB plus cobalt chloride, giving a dark-blue color. These staining sections will be then washed thoroughly (12 hours at 4°C) and incubated with PAP rabbit complex, then revealed in a 0.1% DAB solution giving a yellow-brown coloration.