



ADVANCED TARGETING SYSTEMS

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Anti-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 (15 μ g/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, 200 μ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 μ l 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 200 μ 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 μ l 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 H₂O₂ is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.



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**Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
RABBIT POLYCLONAL
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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 0.1 cacodylate 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 (1/1,000 to 1/5,000), with a monoclonal antibody against conjugated DA (1/1,000 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% hydrogen 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.