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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
MOUSE MONOCLONAL

Catalog Number: AB-T17

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

1. Coating of conjugated L-DOPA (15 μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05 M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001 M, for sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5 g/l of BSA (Acros), 0.05% Tween 20 (Acros) and SMB 0.001 M for one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated L-DOPA antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5 g/l BSA, 10% of glycerol and SMB 0.001 M, 200 μl by well plate (incubating for 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 μl of peroxidase-labeled goat anti-mouse IgG (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5 g/l BSA, 10% of glycerol, 0.5% Tween and SMB 0.001 M, will be applied by well plate (for 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 μl by well plate of a citrate 0.1M/phosphate, 0.2 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, stop the reaction by the addition of 50 μl of 2 M HCl.
9. The optical density will be measured at 492 nm, to obtain the different values (IC 50).

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Example of Immunohistochemistry used to test conjugated L-DOPA conjugate:

Detection of conjugated L-DOPA 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 100ml 0.1M cacodylate buffer containing 5% glutaraldehyde (G) and 0.9% sodium metabisulfite (SMB) pH 7.5.
2. Post fixation: Brains will be removed quickly and post-fixed for 2 hours at 4°C in the same solution.
3. Tissue sectioning: Serial transverse vibratome sections will be cut in Tris- SMB.
4. Application of anti-conjugated antiserum: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB pH 7.5).
   Then, the sections will be washed with in the same solution and incubated in Tris-SMB containing 3% non-specific serum (1h at 4°C):
   Application of anti-conjugated L-DOPA antibodies: The sections will be incubated floating free in plastic wells containing rabbit conjugated L-DOPA antiserum (1/,1000 to 1/5,000) or monoclonal anti conjugated DA antibody (1/,1000 to 1/5,000) for 2 days at 4°C in Tris-SMB buffer pH 7.4.

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: After rinsing 3 times for 10 min in 0.1M Tris buffer containing 0.9% NaCl (Tris-NaCl) pH 7.4, the sections will be incubated with swine anti-rabbit IgG antibodies (Dako) or goat anti-mouse IgG antibodies (Dako). 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 then be incubated (1 hour at 37°C) in Tris-NaCl with a 1/500 dilution of rabbit or mouse peroxidase anti-peroxidase (PAP) complex (Dako).
   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) and 0.01% hydrogen peroxide (Merck).
   The reaction will be stopped by the transfer of the sections in Tris-NaCl buffer.

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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
MOUSE MONOCLONAL

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Example of Immunohistochemistry used to test conjugated L-DOPA conjugate:

Double detection of conjugated L-DOPA and Dopamine (DA) 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).
   Application of anti-conjugated L-DOPA antibodies: Free Floating adjacent sections will be incubated (24h, 4°C) with a monoclonal antibody against G-conjugated L-DOPA (1/,1000 to 1/5,000), with a monoclonal antibody against G-conjugated DA (1/,1000 to 1/5,000), and with both. Antibodies 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.

5. 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 then be 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; 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.

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