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**Anti-Conjugated Octopamine
RABBIT POLYCLONAL**

Catalog Number: AB-T070

Example of ELISA protocol used to test conjugated octopamine:

1. Coating of conjugated octopamine (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) 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. Anti-conjugated octopamine antibodies will be diluted (1/2,000-1/10,000) 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 octopamine:

Detection of conjugated octopamine in dorsal unpaired median neurons innervating the colleterial glands of the female cockroach

1. Dissection: Cockroaches will be dissected under fixative: 1.5% glutaraldehyde in 0.1mol.l⁻¹ cacodylate buffer containing 1% sodium metabisulfite (SMB), pH 7.2.
2. Post fixation: The abdominal nerve cord together with the gland will be removed and placed in the same fixative for 1-1.5h at 20°C.
3. Application of anti-conjugated antiserum: The preparations will be preincubated with 10% normal swine serum in 0.05mol.l⁻¹ Tris-HCl buffer containing 0.5% SMB and 0.5% TritonX-100 (TX) at pH 7.5 for 1h.

Application of anti-conjugated Octopamine antiserum: The octopamine antiserum, diluted 1/1,000 in Tris-HCl-SMB-TX containing 1% swine serum, will be applied to whole mounts for 3 days at 20°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.

4. PAP procedure:
Second antibody: After washing overnight in 0.05mol.l⁻¹ Tris-HCl-TX, pH 7.5, the whole-mounts will be exposed to swine anti-rabbit IgG 1/80 for 12h.
PAP: The tissue will be again washed overnight in Tris-HCl-TX, and a peroxidase/anti-peroxidase (PAP) complex will be applied at a concentration of 1/100 in Tris-HCl-TX with 1% swine serum for 12h.
Revelation: After a final wash, in Tris-HCl buffer, pH7.5 (2x2h), the whole-mounts will be treated for 20min with 4-chloro-1-naphthol (4C1N) using 5mg of 4ICN dissolved in 1ml of methanol and 10ml of Tris-HCl pH7.5 containing 0.05% H₂O₂ (30%). The preparations will be washed in distilled water and mounted in neutral glycerine.