ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Octopamine
RABBIT POLYCLONAL
AB-T070

Example of ELISA protocol used to test conjugated octopamine:

1. Coating of conjugated octopamine (15μg/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, 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 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% H_2O_2 (30%). The preparations will be washed in distilled water and mounted in neutral glycerine.