ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Tyramine
RABBIT POLYCLONAL
AB-T071

Example of ELISA protocol used to test conjugated tyramine:

1. Coating of conjugated tyramine (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 tyramine antibodies 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 protocol used to test conjugated tyramine:

1. Under deep anesthesia, each rat will be perfused through the ascending aorta with 100ml of 0.01M phosphate-buffered saline (PBS) followed by 200ml of fixative containing 2% glutaraldehyde, 1% sodium metabisulfite, and 0.25% picric acid in 0.1M PB.
2. The brain will be removed and cut into several blocks, which will be postfixed in the same fixative for 8h, followed by a rinse for 3 days in PBS containing 20% sucrose and 1% sodium metabisulfite.
3. Sections will be cut with a cryostat in traverse plane.
4. The sections will be incubated in polyclonal antiserum against p-tyramine diluted (1/1,000-1/5,000) in PBS containing 0.3% Triton X-100 and sodium azide ar 4°C for 1 week; biotinylated rabbit IgG (Vector Laboratory, 1/1000) at 4°C for 12h; and avidin-biotin peroxidase complex (Vector Laboratory, 1/1000) at room temperature for 2h.
5. Peroxidase activity will be then revealed in 50mM Tris-HCl buffer (pH 7.6) containing 0.0003% H2O2, 0.01% 3,3'-diaminobenzidine-4HCl (DAB) and 1% nickel ammonium sulfate. The reaction will be terminated by washes in a Tris saline rinse.
6. Sections will be floated onto Tris solution on slides coated with 0.1% chromogelatin, dehydrated and coverslipped. Some serial sections will be stained by polyclonal anti-DA serum for the comparison of anti-p-tyramine staining.