ADVANCED TARGETING SYSTEMS

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Anti-Conjugated 5-Hydroxytryptophol
RABBIT POLYCLONAL
AB-T082

Example of ELISA protocol used to test conjugated 5-hydroxytryptophol:

1. Coating of conjugated 5-hydroxytryptophol (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 5-hydroxytryptophol 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 used to test conjugated 5-hydroxytryptophol:

Example of Protocol Perfusion for Adult male Sprague Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10-1 M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10-1 M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10-1 M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2).
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum.
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).