ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Riboflavin
RAT POLYCLONAL
AB-T142

Example of ELISA protocol used to test conjugated Riboflavine:

1. Coating of conjugated riboflavin (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed riboflavin antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
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.

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Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500 ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
   c) 5,000 ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
   d) 2,000 ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

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 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing Riboflavin antiserum (diluted 1/500–1/1,000; as recommended dilutions).
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-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
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).