Advanced Targeting Systems

Anti-Conjugated Trans-4-Hydroxy-L-Proline
RABBIT POLYCLONAL
AB-T044

Example of Immunochemistry:

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

1. The animals can be deeply anesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1} M, pH 5.4,and with the following solutions:
   a) 200 ml 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) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
   d) Dissect out the organs 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 NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 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 2-10% (variable to adjust) 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 the diluted antiserum (AB-T044 – 1/2000 to 1/5000).
5. Then, the sections will be washed in PBS (30 min).
6. After that incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that 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 H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).