ADVANCED TARGETING SYSTEMS

10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

IMMUNOHISTOCHEMISTY & IMMUNOCYTOCHEMISTRY PROTOCOL FOR "AB-T" ANTIBODIES

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

1. The animals can be deeply anaesthetized, for example with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinize and perfuse via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M PB, pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains are cut on a freezing microtome, infuse the brain in increasing concentrations of sucrose (a first bath of 5% of sucrose in PBS until the brains sink), after that repeat the same process in a solution with a higher level of sucrose (10%, 20%, 25% and finally 30%).
3. Cut serial sections around 50 μm-thick. Keep at 4°C in PBS (0.1 M, pH 7.2) and process for immunostaining.

Immunostaining protocol:

1. In order to avoid possible interference with endogenous peroxidase, treat free-floating sections with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 minutes (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Wash the sections for 20 minutes in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).
3. Pre-incubate for 30 minutes in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1 hour 30 minutes) and overnight at 4°C mixing with a solution containing primary antibodies.
5. Wash for 30 minutes with PBS.
6. Incubate for 60 minutes at room temperature with biotinylated anti-rabbit IgG (Vector) diluted 1/200 in PBS.
7. Wash for 30 minutes with PBS.
8. Incubate sections for 1 hour with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. Wash for 30 minutes with PBS.
10. Wash with Tris-HCl buffer (pH 7.6)(10 minutes).
11. Develop the tissue-bound peroxidase with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Rinse the sections with PBS and coverslip with PBS/Glycerol (1/1).