ADVANCED TARGETING SYSTEMS

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Anti-Conjugated L.Kynurenine Antibody
MOUSE MONOCLONAL
AB-T171

EXAMPLES OF MATERIAL AND METHODS

Example of Immunohistochemistry protocol: Perfusion protocol for Adult male Sprague Dawley (wt ~0.5 kg)

1. The animals can be deeply anaesthetized for example with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   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 4ºC for twelve to sixteen hours.
   e) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first brain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

Around 50 mm-thick serial sections will be obtained, kept at 4º C in PBS (0.1 M, pH 7.2) 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 4º C in the same mixed solution containing anti-conjugated L.Kynurenine antbody (diluted 1/1,000 to 1/5,000; as recommended dilution).
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-mouse 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).

Example of Western blot protocol : Membrane Blocking, Antibody Incubations and Detection

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate membrane with anti-L.Kynurenine antibody diluted 1:1000 in TBS 0.5% Blocker for 2h at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with biotinylated secondary antibody diluted (1/1000-1/2000) in TBS 0.5% Blocker for 2h at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404); TBS = 20mM Tris base, 0.5M NaCl, pH 7.5; TBS Tween = TBS + 0.05% Tween 20