ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Uric Acid
RABBIT POLYCLONAL
AB-T168

Example of Immunochemistry protocol:

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. Heparinized, and perfused 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 in 0.1 M phosphate-buffer (PB), pH 7.2, (two minutes).
   b) 600 ml of cold 4% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (ten minutes).
   c) 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.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain 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 μm-thick serial sections will be obtained, kept at 4°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 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 anti-conjugated Uric acid antibodies (1/1,000-1/5,000).
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) 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) 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 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).

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Example of Western blot protocol:

Membrane blocking, antibodies 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 the membrane with anti-conjugated Uric acid antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours 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 H_2O_2 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

Example of ELISA protocol:

1. Coating of Uric acid(BSA) conjugate (10µg/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 a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 2.5g/l BSA (Acros) and 0.05% Tween 20 (across) (one hour at 37°C).
3. Wash with PBS containing 0.05% of Tween (PBS Tween) (three times).
4. Anti-conjugated Uric acid antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with 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