Advanced Targeting Systems

Anti-Conjugated NO-L-Cysteine
MOUSE MONOCLONAL
AB-T125

Example of ELISA protocol used to test conjugated NO-L-Cystein:

1. Coating of conjugated NO-L-Cystein (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 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated NO-L-Cystein antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 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-mouse (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol 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 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, to obtain the different values (IC 50).

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Example of Immunohistochemistry protocol

Perfusion protocol for Adult male Sprague Dawley (weight around 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 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, (twominutes).
   b) 600 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (tenminutes).
   c) Dissect out the brain and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4ºC for 12 -16 hours.
   d) Before the brains are cut on a freezing microtome, treat the brain in growing concentrations of sucrose (a first solution of 5% of sucrose in PBS until brain sinks), after that, 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 should be obtained, kept at 4ºC 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 are treated with distilled water containing NH3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (another method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. 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% normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4ºC in the same mixed solution containing anti-conjugated NO- L-Cysteine antbody (diluted 1/1,000 to 1/5,000; as recommended dilution).
5. Wash the sections in PBS (30 min).
6. Incubate for 60 min at room temperature with biotinylated anti-mouse immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash the sections in PBS (30 min).
8. Incubate sections for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. Wash the sections in PBS (30 min).
10. Wash with Tris-HCl buffer, pH 7.6 (10 min).
11. Develop tissue-bound peroxidase with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Rinse sections with PBS and coverslip with PBS/Glycerol (1/1).

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Example of Western blot protocol

Membrane Blocking, Antiboby 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-NO-L-Cysteine antibody diluted 1:1000 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