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Anti-Conjugated NO_2-Tyrosine-Diaminopentane
RAT POLYCLONAL
AB-T112

Example of ELISA protocol used to test conjugated NO_2-tyrosine-DP:
Instructions for use: dark conditions

1. Coating of conjugated NO_2-tyrosine-DP (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 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated NO_2-tyrosine-DP antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G 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-rat (Jackson) diluted (1/5,000) in a solution of PBS Tween containing 1g/l of 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.

For research use only, not for use as a diagnostic.

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Example of Immunochemistry protocol used to test conjugated NO_2-tyrosine-DP:
Instructions for use: dark conditions

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

1. The animals can be deeply anaesthetized (for example with urethane 1-1.5g/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) 200ml 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) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) 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.

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 0.15M 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 (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum (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; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h 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).