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**Anti-Conjugated NO₂-Tyrosine-Diaminopentane
RAT POLYCLONAL**

Catalog Number: AB-T112

Example of ELISA protocol used to test conjugated NO₂-tyrosine-DP:

Instructions for use: dark conditions

1. Coating of conjugated NO₂-tyrosine-DP (15 μ g/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 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 NO₂-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, 200 μ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 μ l 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 200 μ l 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 50 μ l of 2M HCl.
9. The optical density will be measured at 492nm.



**ADVANCED
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**Anti-Conjugated NO₂-Tyrosine-Diaminopentane
RAT POLYCLONAL**

Catalog Number: AB-T112

Example of Immunochemistry protocol used to test conjugated NO₂-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) 10⁻¹M, pH 5.4, and with the following solutions:
 - a) 200ml of a solution containing MES 10⁻¹M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10⁻¹M (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 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 0.15M 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) immunoglobulin (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 H₂O₂ using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).