

Anti Nitrotyrosine
(mouse monoclonal IgG)

Catalog # 05-233

Lot # 15590

Immunogen: Nitrated KLH.

Purification: Mouse IgG purified by Protein G-affinity chromatography.

Quantity and Formulation: **100mg** of mouse IgG in **100ml** of 0.1M Tris-glycine, pH 7.2. The amount of antibody was determined by Bradford microtiter protein assay.

Physical Form: Frozen solution.

Storage and Stability: 2 years at -20°C. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and after removing the cap.

References:

1. Beckman, J.S., *et. al.*, Biol. Chem. Hoppe-Seyler **375**: 81-88, 1994.
2. Ohshima, H., *et. al.*, Ed Chem. Tox. **28**: 647-652, 1990.
3. Ischiropoulos, H., *et. al.*, Arch. Biochem. Biophys. **298**: 431-437, 1992.
4. Beckman, J.S., *et. al.*, Nature **364**: 584, 1993.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

Western Immunoblot Analysis: This lot of antibody at 0.5-2 µg/ml detected nitrated protein extracted from chemically-nitrated A431 cells. No reaction was noted with non-nitrated proteins.

Immunoprecipitation: The antibody was unable to immunoprecipitate nitrated BSA.

Immunocytochemistry: 10µg/ml of this lot of antibody gave positive immunostaining for nitrated proteins in A431 cells fixed with 95% ethanol/5% acetic acid.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na₂VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2ng/ml of anti-Nitrotyrosine**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C overnight.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse IgG linked to horseradish peroxidase, 1:2000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunocytochemistry Protocol

1. Plate approximately 200µl of cell suspension into each well of a slide.
2. Incubate 24 hours in a 37°C CO₂ incubator.
3. Wash the cells two times for 10 minutes with PBS with very gentle agitation.
4. Cover the cells with fixative (95% ethanol/5% acetic acid) for 10 minutes at room temperature.
5. Add 100µl peroxyxynitrite (Catalog # 20-107) to each well and incubate for 10 minutes.
6. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
7. Cover the cells with 1% BSA in PBS and incubate for 1 hour at room temperature.
8. Wash the cells with PBS, for 15 minutes.
9. Incubate the cells with **5-10ng/ml anti-Nitrotyrosine** in 1% BSA in PBS and incubate for 1 hour at room temperature.
10. Wash the cells twice with PBS, for 5 minutes.
11. Incubate the cells with a 1:100 dilution of goat anti-mouse IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 1 hour at room temperature.
12. Wash the cells three times with PBS, for 5 minutes.
13. Examine the cells under a fluorescent microscope.