

Anti-Nitrotyrosine

(rabbit polyclonal IgG)

Catalog # 06-284

Lot # 16486

Immunogen: Nitrated KLH.

Formulation: 100mg of immunoaffinity purified rabbit IgG in 255ml of 0.2M Tris-glycine, pH 7.4, 0.15M NaCl, 0.1mM EDTA, 0.05% sodium azide and 10mg/ml BSA. Frozen solution.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

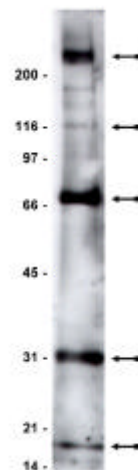
Quality Control Testing

Immunoblot Analysis: 2µg/ml of this lot detected nitrotyrosine molecular weight standards at 14kDa, 31kDa, 66kDa, 116kDa and 200kDa.

Immunocytochemistry: 5µg/ml of this lot detected nitrosylated proteins from human A431 cells pretreated 10 minutes with peroxynitrite (Catalog # 20-107).

Additional Research Applications

Immunohistochemistry: This antibody has been reported to detect nitrotyrosine containing proteins in acutely injured human lung tissue.¹⁻⁵



Immunoblot Analysis

Nitrotyrosine molecular weight standards were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Nitrotyrosine (2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates nitrotyrosine molecular weight standards.

Background: Nitration on the ortho position of tyrosine residues in proteins is a widespread observation in diverse pathological conditions. For example, increased nitration of key tyrosine residues *in vivo* on motor neurons may explain some of the pathology associated with amyotrophic lateral sclerosis. Pretreatment of cells *in vitro* with tetranitromethane can block the phosphorylation of tyrosine kinases by EGF receptor signaling. Nitrotyrosine modification of cellular signaling proteins may play a role in many pathological conditions. Tyrosine nitration may also increase the antigenicity of proteins, thus contributing to diseases of the autoimmune system.

General References:

1. Beckman, J.S., *et al.*, Biol. Chem. Hoppe-Seyler **375**: 81-88 1994.
2. Ohshima, H., *et al.*, Fund. 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.
5. Kooy, N.W., *et al.*, Am. J. Respir. Crit. Care Med. **151**: 1250-1254, 1995.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 1:1 dilution of Laemmli reducing sample buffer and nitrosylated tyrosine molecular weight standards, or a peroxynitrite treated cell lysate, and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Wash the nitrocellulose in PBS-0.05% Tween-20 (PBS-T) for 10 minutes.
3. Wash the nitrocellulose with water twice.
4. 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.
5. Wash the nitrocellulose with water twice.
6. Incubate the nitrocellulose with **2ng/ml of α -Nitrotyrosine** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
7. Wash the nitrocellulose with water five times.
8. Incubate the nitrocellulose in the secondary reagent of choice (1:3000 dilution) in PBS-MLK for 1.5 hours at room temperature with agitation.
9. Wash the nitrocellulose with water five times.
10. Wash the nitrocellulose in PBS-T for 5 minutes.
11. Wash the nitrocellulose with water five times.
12. Use detection method of choice: enhanced chemiluminescence is recommended.

Immunocytochemistry Protocol

1. Plate approximately 200 μ l of cell suspension.
2. Incubate 24 hours in a 37°C CO₂ incubator.
3. Wash the cells three times for 5 minutes with PBS. Do not shake cells.
4. Fix the cells with ethanol:acetic acid [95:5] for 1 minute.
5. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
6. Add 200 μ l peroxynitrite to each well for 10 minutes.
7. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
8. Add 400 μ l of 8% BSA in PBS and incubate for 30 minutes at room temperature.
9. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
10. Incubate the cells with **5ng/ml α -Nitrotyrosine** in 1% BSA in PBS overnight at 4°C.
11. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
12. Incubate the cells with a 1:100 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in PBS for 1.5 hours at room temperature, in the dark.
13. Wash the cells three times with PBS, three times, for 15 minutes in the dark. Do not shake.
14. Mount and examine the cells under a fluorescent microscope.