

## Nitrotyrosine Immunoblotting Control

Catalog # 12-354

Lot # 17249

**Product Description:** A combination of three proteins: nitrated bovine superoxide dismutase (SOD, ~16kDa), nitrated bovine serum albumin (BSA, ~66kDa), and nitrated rabbit muscle myosin (~215kDa). The proteins were nitrated using peroxynitrite<sup>1</sup> (Catalog # 20-107).

**Use:** Add 2.5µl of 2-mercaptoethanol/100µl and boil for five minutes to reduce the preparation. Load 20µl of reduced control per lane for immunoblot analysis. This preparation may be used as a positive control for Upstate Biotechnology's Nitrotyrosine antibodies (Catalog #s 06-284 and 05-233).

**Molecular Weight:** Approximately 16, 66 and 215kDa. Occasionally an approximate 32kDa protein will be seen representing SOD dimer.

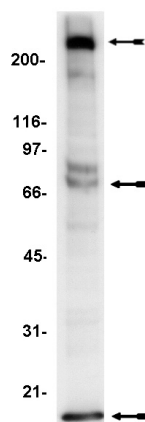
**Blot Applications:** 40 immunoblots, provided in 800µl of PBS, pH 7.4 and non-reducing sample buffer. Frozen solution.

**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 the 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:** 20µl of this lot of Nitrotyrosine Immunoblotting Control was detected in an immunoblot using the polyclonal anti-Nitrotyrosine, (Catalog # 06-284, 2µg/ml). A 1:6000 dilution of goat anti-rabbit IgG conjugated to HRP was used as the secondary antibody in conjunction with enhanced chemiluminescence. These standards are suitable for use with monoclonal anti-Nitrotyrosine (Catalog # 05-233) following the protocol provided on the next page. The arrows indicate the Immunoblot Controls.



**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* in 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.

### References:

1. Ye, Y.Z., *et al.*, Meth Enzymol. **269**: 201-209, 1996.
2. Beckman, J.S., *et al.*, Biol. Chem. Hoppe-Seyler **375**: 81-88, 1994.
3. Ohshima, H., *et al.*, Ed. Chem. Tox. **28**: 647-652, 1990.
4. Ischiropoulos, H., *et al.*, Arch. Biochem. Biophys. **298**: 431-437, 1992.
5. Beckman, J.S., *et al.*, Nature **364**: 584, 1993.
6. Kono, S., *et al.*, Biochem Biophys. Res. Comm. **190**: 283-288, 1993.

**Immunoblot Protocol**  
**(for use with polyclonal antibodies)**

1. Incubate immunoblot control at 37°C for 5 minutes to resuspend the protein. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **20µl of Nitrotyrosine Immunoblotting Control** 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 twice with water.
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 twice with water.
6. Incubate the nitrocellulose with 0.5-2µg/ml of the rabbit polyclonal  $\alpha$ -Nitrotyrosine (Catalog # 06-284) diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
7. Wash the nitrocellulose five times with water.
8. Incubate the nitrocellulose in the secondary reagent of choice (a **goat a-rabbit** HRP conjugated IgG, 1:6000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
9. Wash the nitrocellulose five times with water.
10. Wash the nitrocellulose in PBS-T for 2.5-5 minutes.
11. Wash the nitrocellulose five times with water.
12. Use detection method of choice: enhanced chemiluminescence is recommended.

**Immunoblot Protocol**  
**(for use with monoclonal antibodies)**

1. Incubate immunoblot control at 37°C for 5 minutes to resuspend the protein. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **20µl of Nitrotyrosine Immunoblotting Control** and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Wash the nitrocellulose in TBS, pH 7.5 for 30 minutes.
3. Block the blotted nitrocellulose in TBS, pH 7.5, containing 1% casein (TBS-C), for 2 hours.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose with 2µg/ml of the mouse monoclonal  $\alpha$ -Nitrotyrosine (Catalog # 05-233) diluted in TBS-C for 48 hours.
6. Wash the nitrocellulose for 30 minutes with 0.02% PBS-Tween 20 (PBS-T).
7. Wash the nitrocellulose twice with water.
8. Incubate the nitrocellulose in the secondary reagent of choice (a **goat a-mouse** HRP conjugated IgG, 1:2000 dilution was used) in TBS-C for 90 minutes at room temperature with agitation.
9. Wash the nitrocellulose five times with water.
10. Wash the nitrocellulose in PBS-T for 2.5-5 minutes.
11. Wash the nitrocellulose five times with water.
12. Use detection method of choice: enhanced chemiluminescence is recommended.