



cell signaling solutions

## Certificate of Analysis

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### Nitrotyrosine Immunoblotting Control

Catalog # 12-354

Lot # 23709

**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. A dimer of SOD is occasionally observed at ~32kDa. The proteins were nitrated using peroxynitrite (Catalog # 20-107).<sup>1</sup>

**Use:** Dilute 1:1 with Reducing Sample Buffer and boil for five minutes to reduce the preparation. Load 20 $\mu$ l of reduced control per lane for immunoblot analysis. This preparation may be used as a positive control for Upstate's Nitrotyrosine antibodies (Catalog # 06-284 and 05-233).

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

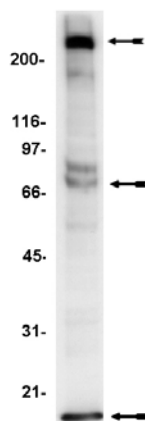
**Blot Applications:** 40 immunoblots, provided in 400 $\mu$ l of PBS, pH 7.4. Protein concentration: nitrated-myosin 16 $\mu$ g/ml; nitrated-BSA 57 $\mu$ g/ml; nitrated-SOD 33 $\mu$ g/ml. Total protein concentration: 105 $\mu$ g/ml. Frozen solution.

**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:** 10 $\mu$ l of this lot of Nitrotyrosine Immunoblotting Control was detected in an immunoblot using 0.5-2 $\mu$ g/ml polyclonal anti-Nitrotyrosine (Catalog # 06-284). A 1:5000 dilution of goat anti-rabbit HRP conjugated IgG (Catalog # 12-348) was used as the secondary antibody in conjunction with enhanced chemiluminescence. These standards were also tested using 0.5-2 $\mu$ g/ml monoclonal anti-Nitrotyrosine (Catalog # 05-233). A 1:2000 dilution of goat anti-mouse HRP conjugated IgG (Catalog # 12-349), was used as the secondary antibody. The arrows indicate the Immunoblot Controls.



### References:

1. Balafanova, Z., *et al.*, *J. Biol. Chem.* **277**: 15021-15027.
2. Ye, Y.Z., *et al.*, *Meth Enzymol.* **269**: 201-209, 1996.
3. Beckman, J.S., *et al.*, *Biol. Chem. Hoppe-Seyler* **375**: 81-88, 1994.
4. Ohshima, H., *et al.*, *Ed. Chem. Tox.* **28**: 647-652, 1990.
5. Ischiropoulos, H., *et al.*, *Arch. Biochem. Biophys.* **298**: 431-437, 1992.
6. Beckman, J.S., *et al.*, *Nature* **364**: 584, 1993.

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

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **10 $\mu$ l of Nitrotyrosine Immunoblotting Control** diluted 1:1 with RSB 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 (Catalog # 20-200), (PBS-MLK) for 20 minutes to 1 hour at room temperature with constant agitation.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose with 0.5-2 $\mu$ g/ml of rabbit polyclonal anti-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 anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 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. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **20 $\mu$ l of a 1:1 dilution of RSB: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 for 10 minutes.
3. Wash the nitrocellulose twice with water.
4. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog# 20-200), (PBS-MLK) for 20-60 minutes at room temperature with constant agitation.
5. Incubate the nitrocellulose with 0.5-2 $\mu$ g/ml of mouse monoclonal anti-Nitrotyrosine (Catalog # 05-233), diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
6. Wash the nitrocellulose twice with water.
7. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
8. Wash the nitrocellulose with water twice.
9. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
10. Rinse the nitrocellulose in 4-5 changes of water.
11. Use detection method of choice (enhanced chemiluminescence was used).