

## Anti-Nitrotyrosine, agarose conjugate

(mouse monoclonal IgG)

Catalog # 16-163

Lot # 18462

**Product Description:** Anti-Nitrotyrosine monoclonal antibody, clone 1A6 (Catalog # 05-233) cross-linked to protein G agarose by dimethylpimelimidate.

**Formulation:** 100µg of monoclonal anti-Nitrotyrosine covalently coupled to 50µl of protein G agarose beads. Provided as a 50% slurry for a final volume of 100ml. Beads are suspended in phosphate buffered saline (PBS), pH 7.4 containing 0.05% sodium azide. Liquid suspension.

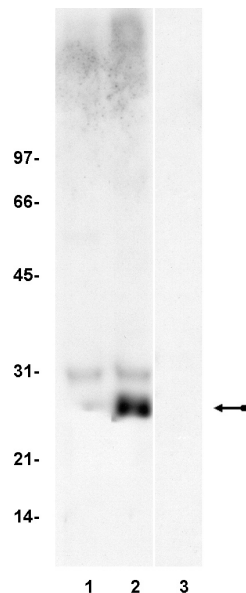
**Note:** It is recommended to wash the agarose beads with appropriate buffer prior to use to remove sodium azide.

**Storage and Stability:** Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge the original vial prior to removing the cap.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoprecipitation:** 10-15µl of a 50% gel slurry of this lot immunoprecipitated nitrated MnSOD from peroxynitrite-treated rat heart lysate as detected by subsequent immunoblot analysis using 4µg/ml anti-MnSOD (Catalog # 06-984).



**Immunoprecipitation/Immunoblot:** Untreated (lane 1) or peroxynitrite-treated (lanes 2 & 3) rat heart lysate was incubated with 15µl anti-nitrotyrosine, agarose conjugate (lanes 1 & 2) or a non-specific mouse IgG-agarose (lane 3). The immunoprecipitated proteins were resolved with electrophoresis, transferred to nitrocellulose and incubated with anti-MnSOD. Arrow indicates nitrated MnSOD.

### Immunoprecipitation/Immunoblot Protocol

1. Before beginning the immunoprecipitation, dilute the cell or tissue lysate to roughly  $1\mu\text{g}/\mu\text{l}$  total cell protein in a microcentrifuge tube with PBS.
2. Add **10ml gel slurry of anti-Nitrotyrosine, agarose conjugate** to  $500\mu\text{g}$ -1mg cell lysate.
3. Gently rock the reaction mixture at  $4^{\circ}\text{C}$  overnight.
4. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at  $14,000 \times g$ ), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
5. Resuspend the agarose beads in  $60\mu\text{l}$  2X Laemmli sample buffer.
6. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads using a microcentrifuge pulse and perform SDS-PAGE and immunoblot analysis on a sample of the supernatant. Transfer the proteins to nitrocellulose and wash the blotted nitrocellulose twice with water.
7. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at  $20$ - $25^{\circ}\text{C}$  with constant agitation.
8. Incubate the nitrocellulose with  $4\mu\text{g}/\text{ml}$  of anti-MnSOD (Catalog # 06-984), diluted in freshly prepared PBS-MLK overnight with agitation at  $4^{\circ}\text{C}$ .
9. Wash the nitrocellulose twice with water.
10. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** HRP conjugated IgG, 1:3000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
11. Wash the nitrocellulose with water twice.
12. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
13. Rinse the nitrocellulose in 4-5 changes of water.
14. Use detection method of choice (enhanced chemiluminescence was used).