

Certificate of Analysis

Anti-Nitrotyrosine, clone 1A6, agarose conjugate

(mouse monoclonal IgG)

Catalog # 16-163

Lot # 30242

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 100µl. Beads are suspended in phosphate buffered saline (PBS), pH 7.4 containing 0.05% sodium azide. Liquid suspension.

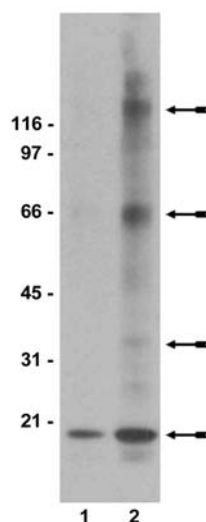
Note: It is recommended to wash the agarose beads with appropriate buffer prior to use to remove sodium azide.

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: 40µl of a 50% gel slurry of this lot immunoprecipitated nitrated proteins from Nitrotyrosine Immunoblotting Control (Catalog # 12-354) as detected by subsequent immunoblot analysis using 1µg/ml anti-Nitrotyrosine (Catalog # 06-284).



Immunoprecipitation/Immunoblot: Representative blot from a previous lot. Nitrated proteins were incubated with anti-Phosphotyrosine (4G10), agarose conjugate (Catalog # 16-101) (lane 1) or anti-Nitrotyrosine, agarose conjugate (lane 2). The immunoprecipitated proteins were analyzed by immunoblotting with anti-Nitrotyrosine. Arrows indicate nitrated standards.

Application References:

1. Gaut, J. P., *et al.*, *J. Clin. Invest.* **109**: 1311-9, 2002.

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 **40 μl gel slurry of anti-Nitrotyrosine, agarose conjugate** to 400 μl Nitrotyrosine Immunoblotting Control (Catalog # 12-354).
3. Gently rock the reaction mixture for 2 hrs. at room temperature or overnight at 4°C.
4. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x 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 40 μl 2X Laemmli sample buffer.
6. Store the beads frozen for future analysis or boil the beads for 5 minutes.
7. Collect the beads after boiling using a microcentrifuge pulse. Load 10-20 μl per lane.
8. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.
9. Transfer the proteins to nitrocellulose and wash the blotted nitrocellulose twice with water.
10. Wash the nitrocellulose in PBS-0.05% Tween 20 for 10 minutes.
11. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 60 minutes at room temperature with constant agitation.
12. Incubate the nitrocellulose with 1-2 $\mu\text{g}/\text{ml}$ of anti-Nitrotyrosine (Catalog # 06-284), diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
13. Wash the nitrocellulose twice with water.
14. 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.
15. Wash the nitrocellulose twice with water.
16. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
17. Rinse the nitrocellulose in 4-5 changes of water.
18. Use detection method of choice (enhanced chemiluminescence was used).