



cell signaling solutions

Certificate of Analysis

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Anti-Nitrotyrosine, clone 1A6

(mouse monoclonal IgG_{2bκ})

Catalog # 05-233

Lot # 27637

Immunogen: Nitrated KLH. Clone 1A6.

Formulation: 100µg of protein G purified mouse IgG_{2bκ} in 100µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl with 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 2 years 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**

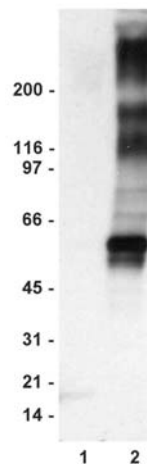
Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected nitrated proteins in a lysate prepared from peroxynitrite-treated A431 cells. A previous lot detected nitrotyrosine immunoblotting controls (Catalog # 12-354). No reaction was noted with non-nitrated proteins.

Immunocytochemistry: 5-10µg/ml of this lot showed positive immunostaining for nitrated proteins in peroxynitrite-treated (24mM for 5 minutes) A431 cells fixed with 95% ethanol/5% acetic acid.

Additional Research Applications

Immunoprecipitation: This clone has been conjugated to agarose for easy immunoprecipitations (Catalog # 16-163). See also reference 1.



Immunoblot Analysis

Representative blot from a previous lot. A431 cell lysate was treated without (lane 1) or with (lane 2) Peroxynitrite (Catalog # 20-107). Nitrated proteins were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-nitrotyrosine (0.5µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

References:

1. MacMillan-Crow, L.A., *et al.*, *Proc. Natl. Acad. Sci. USA* **93**: 11853-11858, 1996.
2. Beckman, J.S., *et al.*, *Biol. Chem. Hoppe-Seyler* **375**: 81-88, 1994.
3. Beckman, J.S., *et al.*, *Nature* **364**: 584, 1993.
4. Ischiropoulos, H., *et al.*, *Arch. Biochem. Biophys.* **298**: 431-437, 1992.
5. Ohshima, H., *et al.*, *Ed. Chem. Tox.* **28**: 647-652, 1990.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) 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 **0.5-2µg/ml of anti-Nitrotyrosine**, 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).

Immunocytochemistry Protocol

1. Plate approximately 200µl of cell suspension into each well of a slide.
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 200µl peroxyntirite (at 24mM) to the positive control wells for 5 minutes.
7. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
8. Cover the cells with 400µl of 1% BSA in PBS and incubate for 60 minutes at room temperature.
9. Wash the cells twice with PBS for 15 minutes per wash.
10. Incubate the cells with **5-10µg/ml anti-Nitrotyrosine** in 1% BSA in PBS overnight at 4°C or 2 hours at room temperature.
11. Wash the cells twice with PBS for 15 minutes.
12. Incubate the cells in the dark, with a 1:100 dilution of goat anti-mouse IgG fluorescein conjugated secondary antibody in PBS for 1-1.5 hours at room temperature.
13. Wash the cells three times with PBS for 15 minutes.
14. Examine the cells under a fluorescent microscope.