

## Certificate of Analysis

### Anti-Nitrotyrosine, clone 1A6

(mouse monoclonal IgG<sub>2bκ</sub>)

Catalog # 05-233

Lot # DAM1430000

**Immunogen:** Nitrated KLH. Clone 1A6.

**Formulation:** 100µg of protein G purified mouse IgG<sub>2bκ</sub> in 100µl of storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide). Frozen at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C.

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

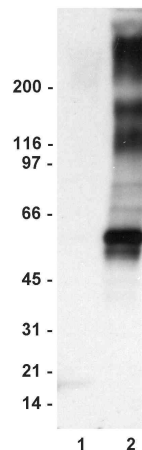
### Quality Control Testing

**Immunoblot Analysis:** 0.5-1µg/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 (5-10mM 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 Na<sub>3</sub>VO<sub>4</sub>; 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 CO<sub>2</sub> 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 peroxyxynitrite (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.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals."