

# Anti-Nitrotyrosine, clone 1A6

Monoclonal Antibody

Cat. # 05-233

Lot # DAM1594191

pack size: 100 µg

Store at -20°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IC, IH	WR	IgG2bκ	N/A	M	See Below	N/A

### Background

Nitrotyrosine has been identified as an indicator of cell damage and inflammation, as well as of the production of NO. It is believed that measuring the concentration of nitrotyrosine will serve as a marker for damage caused by NO in the cell. Previous studies have shown that nitrotyrosine has been found in inflammatory conditions such as atherosclerotic plaques and rheumatoid arthritis, among others.

### Presentation

Purified mouse IgG2bκ in storage buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide. Frozen at -20°C.

### Concentration

1 mg/mL

### Specificity

Recognizes nitrated protein

### Species Cross-reactivity

Predicted to react with most common vertebrate species

### Immunogen

Nitrated KLH, clone 1A6

### Molecular Weight

Predicted MW for nitrated-BSA: 85 kDa

### Method of Purification

Protein G purified

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

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.

### Control

Immunohistochemistry: Human Alzheimer disease brain sections (Methacarn fixed), mouse liver

Immunoblotting: Nitrated-BSA

Indirect ELISA: Nitrated-BSA

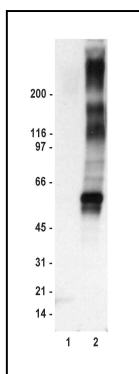
Indirect Immunofluorescence: A549 cells metabolically labeled with 3-nitrotyrosine.

### Quality Control Testing

Routinely evaluated on peroxynitrite-treated A431 cells; no reaction was noted with non-nitrated proteins.

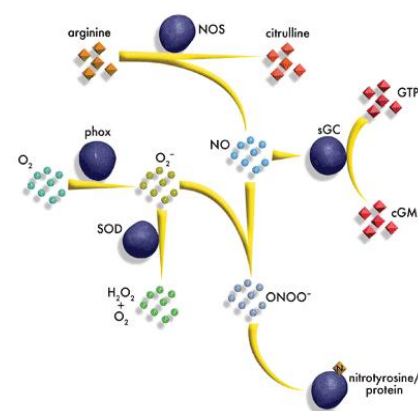
### Western Blot Analysis:

0.5-2 µg/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.



### Western Blot Analysis:

Representative lot data. 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 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.



### References

- Gow, A.J., et al. (2004). *Am J Physiol Lung Cell Mol Physiol.* 287(2):L262-8.
- Balafanova, Z., et al. (2002). *J Biol Chem.* 277:15021-7.
- Bebok, Z., et al. (2002). *J Biol Chem.* 277:43041-9.
- Zou, M. H., et al. (2002). *J Clin Invest.* 109:817-26.
- Torreilles, J. and Romestand, B. (2001). *BMC Immunol.* 2: 1.

### Additional Research Applications

#### Immunocytochemistry:

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

#### Immunoprecipitation:

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

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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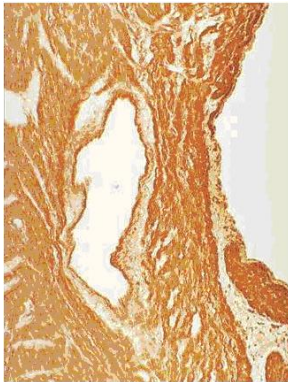
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**ADDITIONAL RESEARCH APPLICATIONS**



Immunohistochemistry:

Viral infected human heart stained with anti-Nitrotyrosine, clone 1A6 (1 mg/mL).

**PROTOCOL**

**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM 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 0.5-2 µg/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**

1. Plate approximately 200 µL 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 200 µL 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 400 µL 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 5-10 µg/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.

**RELATED PRODUCTS (specific)**

cat #	description
AB5900	Anti-Nitric Oxide-Tyramine
AB5530	Anti-Nitric Oxide-Tyrosine
AB5532	Anti-Nitrotyrosine
06-284	Anti-Nitrotyrosine
AB5411	Anti-Nitrotyrosine
16-163	Anti-Nitrotyrosine, clone 1A6, agarose conjugate
16-226	Anti-Nitrotyrosine, clone 1A6, Alexa Fluor® 488 conjugate
16-227	Anti-Nitrotyrosine, clone 1A6, Alexa Fluor® 555 conjugate
16-207	Anti-Nitrotyrosine, clone 1A6, HRP conjugate
MAB5404	Anti-Nitrotyrosine, clone 2A8.2
16-216	Anti-Nitrotyrosine, Cy5.5 conjugate
17-376	Nitrotyrosine ELISA
12-354	Nitrotyrosine Immunoblotting Control
12-349	Goat Anti-Mouse IgG
AP124P	Goat anti-Mouse IgG, Peroxidase Conjugated, H+L

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	Immobilon Western Chemillum HRP Substrate 100 mL
17-373	Spray & Glow™ ECL WB Detection System 1 ea
2060	Re-Blot Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker Membrane Blocking Agent 175G

antibodies Multiplex products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

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