

Datasheet for 200-301-A98**Nitrotyrosine Antibody****Overview**

Description:	Anti-Nitrotyrosine (MOUSE) Monoclonal Antibody - 200-301-A98
Item No.:	200-301-A98
Size:	100 µg
Applications:	IHC, WB
Reactivity:	Broad
Host Species:	Mouse

Product Details

Background:	Protein tyrosine nitration results in a post-translational modification that is increasingly receiving attention as an important component of nitric oxide signaling (2). While multiple nonenzymatic mechanisms are known to be capable of producing nitrated tyrosine residues, most tyrosine nitration events involve catalysis by metalloproteins such as myeloperoxidase, eosinophil peroxidase (3), myoglobin, the cytochrome P-450s, superoxide dismutase and prostacyclin synthase. Nitrotyrosine may also serve as a biomarker for the effects of reactive nitrogen oxides, based on tyrosine residues becoming nitrated in proteins at sites of inflammation-induced tissue injury (1). The presence of nitro tyrosine-containing proteins has shown high correlation to disease states such as atherosclerosis, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (4).
Synonyms:	Protein tyrosine nitration, 3-(4-hydroxy-3-nitrophenylacetamido) propionic acid, Nitro tyrosine Antibody, 3-Nitrotyrosine Antibody
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	39B6
Format:	IgG2a

Target Details

Reactivity:	Broad
Immunogen Type:	Native Protein

Immunogen:	This Protein G purified monoclonal antibody was prepared using conventional hybridoma technology after repeated immunizations with 3-(4-Hydroxy-3-nitrophenyl acetamido) propionic acid - BSA conjugate.
Purity/Specificity:	This Protein G purified monoclonal antibody reacts with proteins containing 3-nitrotyrosine moieties and does not detect non-nitrated tyrosine residues. This antibody is known to react with nitrated tyrosine residues from human, mouse, rat and dog sources. Reactivity with nitrotyrosine from other sources is also expected.

Application Details

Tested Applications:	IHC, WB
Application Note:	This Protein G purified antibody has been tested for use in ELISA, immunoprecipitation, immunohistochemistry-P, and western blotting. Specific conditions for reactivity should be optimized by the end user. Approximately 0.7 µg of antibody is sufficient to detect 5 µg of SIN-1 treated BSA by Western blotting using HRP Gt-a-Mouse IgG (p/n 610-103-121) and chemiluminescent substrate (p/n PICOMAX-110).
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IHC:	1:200 - 1:1,000
WB:	1:500 - 1:2,000

Formulation

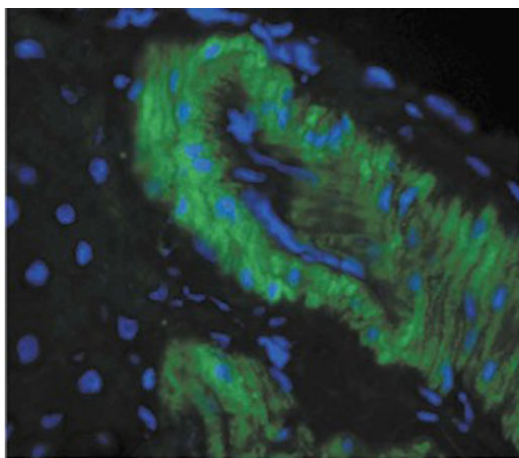
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.09% (w/v) Sodium Azide
Stabilizer:	50% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Expiration:

Expiration date is one (1) year from date of receipt.

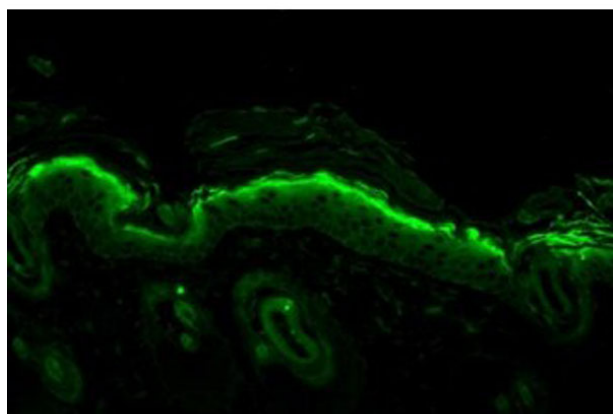
Images**Immunofluorescence Microscopy**

Immunohistochemistry of Mouse Anti-Nitrotyrosine Monoclonal Antibody.

Tissue: rat liver tissue.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:1000.

Secondary Antibody: FITC Goat Anti-Mouse (green).

**Immunofluorescence Microscopy**

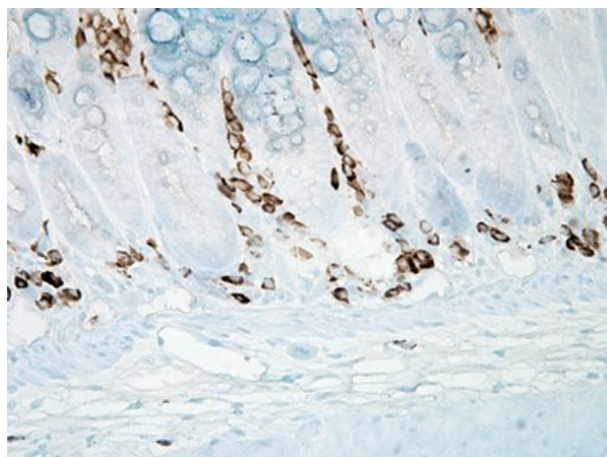
Immunohistochemistry of Mouse Anti-Nitrotyrosine Monoclonal Antibody.

Tissue: mouse backskin from transgenic mice.

Fixation: Bouin's Fixative and paraffin-embedded.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:100 for 1 hour at RT.

Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:50 for 1 hour at RT.



Immunohistochemistry

Immunohistochemistry of Mouse Anti-Nitrotyrosine Monoclonal Antibody.

Tissue: mouse inflamed colon.

Fixation: Formalin with anti-microbial.

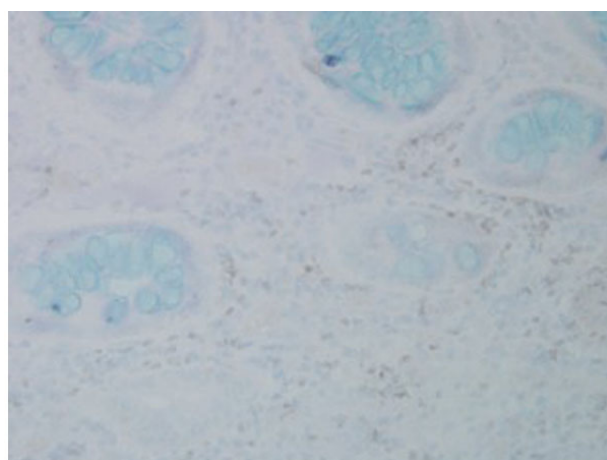
Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:1,000,000 for 12 hours at 4°C.

Secondary Antibody: Biotin Goat Anti-Mouse at 1:2000 for 1 hour at RT.

Counterstain: Mayer Hematoxylin (purple/blue) nuclear stain at 200 µl for 2 minutes at RT.

Magnification: 40x.

This image was produced using an amplifying IHC wash buffer. The antibody has therefore been diluted more than is recommended for other applications.



Immunohistochemistry

Immunohistochemistry of Mouse Anti-Nitrotyrosine Monoclonal Antibody.

Tissue: human colon carcinoma.

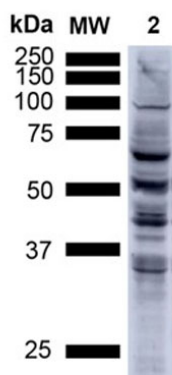
Fixation: Formalin.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:25,000 for 12 hours at 4°C.

Secondary Antibody: Biotin Goat Anti-Mouse at 1:2000 for 1 hour at RT.

Counterstain: Mayer Hematoxylin (purple/blue) nuclear stain at 200 µl for 2 minutes at RT.

Magnification: 40x.



Western Blot

Western Blot of Anti-Nitrotyrosine.

Load: 15 ug. Lane 1: MW ladder. Lane 2: Human A549 Cells.

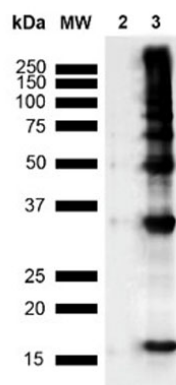
Block: 5% Skim Milk in TBST.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:1000 for 2.5 hours at RT.

Secondary Antibody: Goat anti-mouse IgG HRP at 1:1000 for 1 hour at RT.

Color Development: Chemiluminescent for HRP for 5 min in RT.

Predicted/Observed Size: Multiple Bands.



Western Blot

Western Blot of Anti-Nitrotyrosine.

Lane 1: MW Ladder. Lane 2: hASYN Monomer (3.84 ug). Lane 3: Nitrosylated hASYN (3.84 ug).

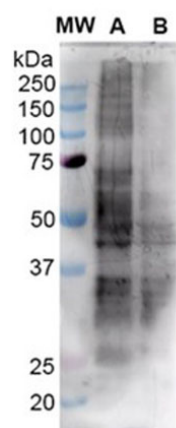
Block: 5% Skim Milk in TBST.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody at 1:1000 for 2 hours at RT.

Secondary Antibody: Goat anti-mouse IgG HRP at 1:4000 for 2 hour at RT.

Color Development: Chemiluminescent for HRP for 5 min in RT.

Predicted/Observed Size: Multiple Bands.



Western Blot

Western Blot of Anti-Nitrotyrosine.

Lane 1: MW Ladder. Lane A: Nitrosylated-HEK293 (15uL).

Lane B: HEK293 (15 ug).

Block: 5% Skim Milk in TBST.

Primary Antibody: Mouse Anti-Nitrotyrosine Monoclonal Antibody diluted in 1.5% BSA and TBST for 1 hours at RT.

Secondary Antibody: Goat anti-mouse IgG HRP at 1:4000 for 1 hour at RT.

Predicted/Observed Size: Multiple Bands.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.