Nitrotyrosine Antibody

Monoclonal Mouse IgG_3 Clone # 306507

Catalog Number: MAB3248

DESCRIPTION

Specificity	Detects Nitrotyrosine adducts on proteins in Western blots. It does not cross-react with phosphotyrosine or 4-hydroxynonenal adducts. Unfixed cells, tissues, and proteins can be treated with Peroxynitrite (Catalog # AR006) for use as positive controls with this antibody.
Source	Monoclonal Mouse IgG_3 Clone # 306507
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Nitrotyrosine-modified KLH
Formulation	Lyophilized from a 0.2 µm filtered solution in TBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

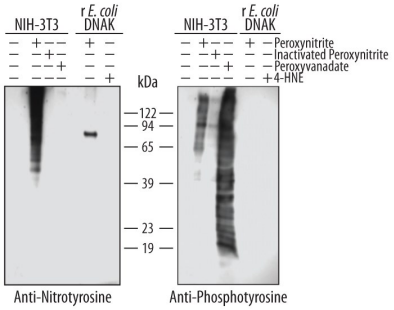
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

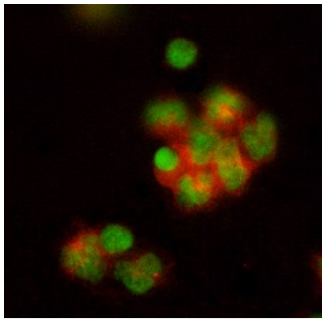
DATA

Western Blot



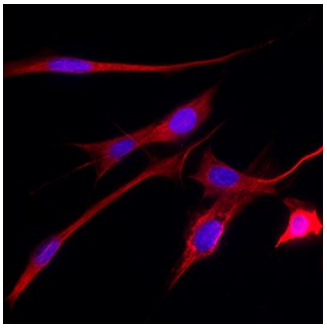
Detection of Nitrotyrosine by Western Blot. Western blot shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 3 mM peroxynitrite, 3 mM inactivated peroxynitrite, or 100 µM peroxyvanadate for 1 hour and recombinant *E. coli* DNAK treated with 1 mM 4-hydroxynonenal or 1 mM peroxyynitrite for 1 hour. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Nitrotyrosine Monoclonal Antibody (Catalog # MAB3248), followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). The lysates were also probed with Phospho-Tyrosine Monoclonal Antibody (Catalog # MAB1676). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Immunocytochemistry



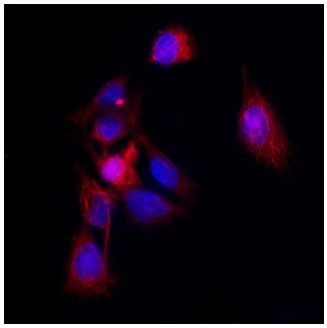
Nitrotyrosine in Human PBMCs. Nitrotyrosine was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using 25 µg/mL Mouse Anti-Nitrotyrosine Monoclonal Antibody (Catalog # MAB3248) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained (green). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunocytochemistry



Detection of Nitrotyrosine in A549 human lung carcinoma cells Nitrotyrosine was detected in immersion fixed A549 human lung carcinoma cells using Mouse Anti-Nitrotyrosine Monoclonal Antibody (Catalog # MAB3248) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to Cytoplasmic. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunocytochemistry



Detection of Nitrotyrosine in HepG2 cells Nitrotyrosine was detected in immersion fixed HepG2 human hepatocellular carcinoma cells using Mouse Anti-Nitrotyrosine Monoclonal Antibody (Catalog # MAB3248) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to Cytoplasmic. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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Nitrotyrosine Antibody

Monoclonal Mouse IgG_3 Clone # 306507

Catalog Number: MAB3248

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

3-Nitrotyrosine is formed when tyrosine is reacted with peroxynitrite. Since peroxynitrite is formed from nitric oxide and superoxide anion, nitrotyrosine adducts on proteins have been used as markers of oxidative cellular damage and macrophage activation. Elevated nitrotyrosine immunoreactivity has been found in inflammation, osteoarthritis, neurodegenerative diseases, and ischemic damage to the heart and brain.

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