

DESCRIPTION	
Species Reactivity	Human/Mouse/Rat
Specificity	Detects human iNOS. By using synthetic peptides, the epitope recognized by this antibody has been mapped to aa 781-798 of human iNOS. The corresponding sequence of mouse iNOS is identical.
Source	Monoclonal Mouse IgG ₁ Clone # 2D2-B2
Purification	Protein A or G purified from ascites
Immunogen	Recombinant human iNOS Pro781-His798 Accession # P35228
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied 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
Immunohistochemistry	8-25 µg/mL	See Below
Simple Western	10 µg/mL	See Below

DATA

Western Blot

Detection of Human iNOS by Western Blot. Western blot shows lysates of DLD clone 2C2 human colon adenocarcinoma cell line untreated (-) or treated (+) with Recombinant Human IL-1β, Recombinant Human TNF-α, and Recombinant Human IFN-γ (Catalog #201-LB, 210-TA, 285-IF) for 24 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat iNOS Monoclonal Antibody (Catalog # MAB9502), followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for iNOS at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 6.

Western Blot

Detection of Mouse and Rat iNOS by Western Blot. Western blot shows lysates of RAW 264.7 mouse monocyte/macrophage cell line and NR8383 rat alveolar macrophage cell line untreated (-) or treated (+) with 10 µg/mL LPS for 4 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat iNOS Monoclonal Antibody (Catalog # MAB9502) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for iNOS at approximately 125 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry

iNOS in Human Brain. iNOS was detected in immersion fixed paraffin-embedded sections of human brain (medulla) using Mouse Anti-Human/Mouse/Rat iNOS Monoclonal Antibody (Catalog # MAB9502) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to neuronal cell bodies. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western

Detection of Mouse iNOS by Simple Western™. Simple Western lane view shows lysates of RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with LPS, loaded at 0.2 mg/mL. A specific band was detected for iNOS at approximately 136 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human/Mouse/Rat iNOS Monoclonal Antibody (Catalog # MAB9502). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 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

Inducible nitric oxide synthase (iNOS) is a widely expressed, approximately 130 kDa enzyme that catalyzes the conversion of L-arginine to nitric oxide (NO) plus citrulline. NO is a bioactive mediator that plays an important role in hemodynamics by inducing vascular smooth muscle relaxation. iNOS is constitutively expressed in the kidney. In other tissues, it is inducibly expressed during inflammation, oxidative stress, and hyperglycemia. iNOS activity is elevated in a variety of disease states including atherosclerosis, heart failure, sepsis, solid tumors, and type 2 diabetes. Increased production of NO mediates acute kidney injury, TNF-alpha induced muscle wasting, and the increased radiosensitivity of hypoxic tumor cells. Within amino acids 781-798, human iNOS shares 89% and 72% aa sequence identity with mouse and rat iNOS, respectively.