

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human nNOS in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG ₁ Clone # 85340
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human nNOS Ser218-Ser1434 Accession # P29475
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.

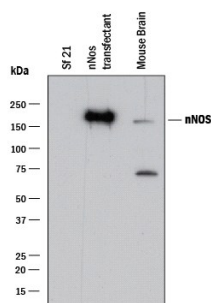
APPLICATIONS

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
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below

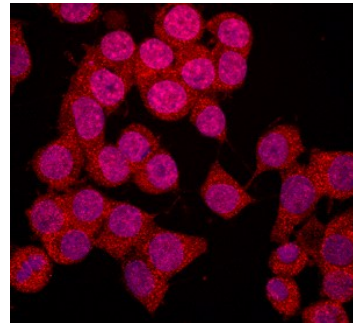
DATA

Western Blot



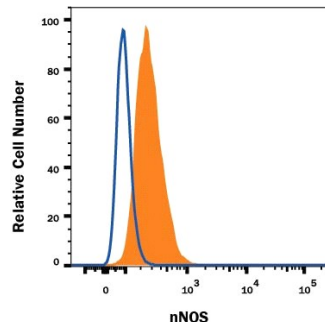
Detection of Human and Mouse nNOS by Western Blot. Western blot shows lysates of Sf 21 *S. frugiperda* insect ovarian cell line either mock transfected or transfected with human nNOS, and mouse brain tissue. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse nNOS Monoclonal Antibody (Catalog # MAB24161) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for nNOS at approximately 160 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



nNOS in Neuro-2A Mouse Cell Line. nNOS was detected in immersion fixed Neuro-2A mouse neuroblastoma cell line using Mouse Anti-Human/Mouse nNOS Monoclonal Antibody (Catalog # MAB24161) at 10 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 nuclei, cytoplasm and cell membrane. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Intracellular Staining by Flow Cytometry



Detection of nNOS in Neuro-2A Mouse Cell Line by Flow Cytometry. Neuro-2A mouse neuroblastoma cell line was stained with Mouse Anti-Human/Mouse nNOS Monoclonal Antibody (Catalog # MAB24161, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for [Staining Intracellular Molecules](#).

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

nNOS is one of three NOS enzymes that catalyze the oxidation of L-arginine to L-citrulline and nitric oxide. nNOS exists as homodimers containing a cytochrome P450-like prosthetic heme group in the N-terminal half. It also has a tightly bound FAD and FMN group in the C-terminal half. At least 4 isoforms of human nNOS are known. Human nNOS shares about 55% amino acid sequence identity with eNOS and iNOS. It also shares 96% sequence identity with mouse or rat nNOS.