

## Background

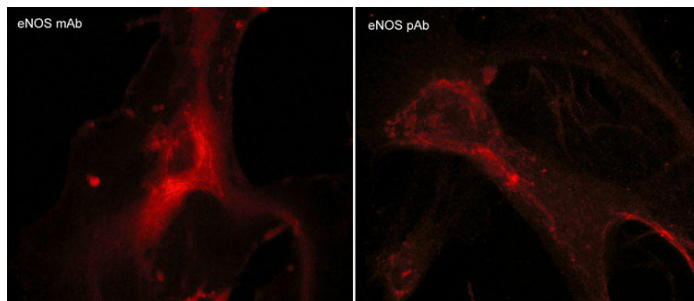
Nitric oxide (NO) has a broad range of biological activities and is implicated in signaling pathways in phylogenetically diverse species. Nitric oxide synthases (NOS), the enzymes responsible for synthesis of NO, are homodimers whose monomers are themselves two fused enzymes: a cytochrome reductase and a cytochrome that requires three cosubstrates (L-arginine, NADPH, and oxygen) and five cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin, and heme). Several distinct NOS isoforms are produced from three distinct genes. The inducible form of NOS, iNOS (NOS-II), is Ca<sup>2+</sup> independent and is expressed in a broad range of cell types, and two constitutive Ca<sup>2+</sup>/CaM-dependent forms of NOS: nNOS (bNOS, NOS-I) identified in neurons and eNOS (ecNOS, NOS-III) identified in endothelial cells. Regulation of eNOS activity occurs through phosphorylation at multiple sites. Phosphorylation of Ser-633 (mouse Ser-632) in the FMN binding domain increases eNOS activity and may be important for the maintenance of NO synthesis after initial activation by Ca<sup>2+</sup> flux and Ser-1177 phosphorylation.



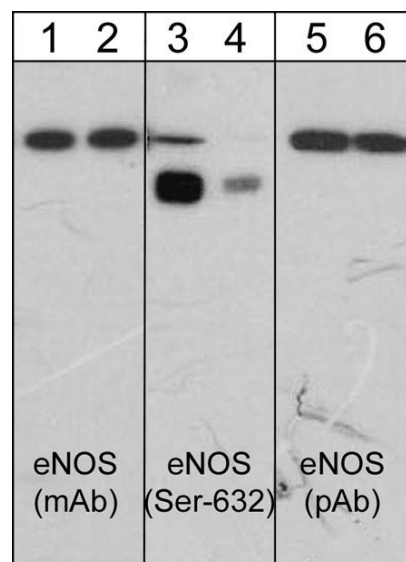
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## Background References

- Xie, Q.W. et al. (1992) Science 256:225.  
Musicki, B. et al. (2005) Proc. Natl. Acad. Sci.102(33):11870.  
Mount, P.F. et al. (2007) J Mo.l Cell. Cardiol. 42(2):271.



Immunocytochemical labeling of endothelial nitric oxide synthase (eNOS) in paraformaldehyde-fixed and NP-40-permeabilized human umbilical vein endothelial cells. The cells were labeled with mouse monoclonal eNOS (NM2211) and rabbit polyclonal eNOS (NP2281) antibodies, then the antibodies were detected using appropriate secondary antibodies conjugated to Cy3.



Western blot analysis of human umbilical vein endothelial cells before (lanes 1, 3, 5) and after (lanes 2, 4, 6) treatment with lambda phosphatase. The blots were probed with anti-endothelial Nitric Oxide Synthase (eNOS) monoclonal antibody (lanes 1 & 2), anti-eNOS (Ser-632) phospho-specific antibody (lanes 3 & 4), and anti-eNOS polyclonal antibody (lanes 5 & 6).

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**Immunogen****Uniprot ID: P70313**

Clone (M221) was generated from a recombinant mouse eNOS protein that included amino acids residues in the C-terminal region. This sequence is conserved in human and rat eNOS, and has low homology to other NOS family members.

**Buffer and Storage**

Mouse monoclonal antibody purified with protein A chromatography is supplied in 100µl phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at -20°C. Stable for 1 year.

**Applications**

WB	1:1000
ELISA	1:2000
ICC	1:50
IHC	1:200

**Species Reactivity**

Hu, Rt, Ms

**Isotype: IgG1**

End user should determine optimal dilution for their particular applications and experiments.  
Western blot membranes were incubated with diluted antibody in 5% non-fat milk, Tris buffer, 0.04% Tween20 for 1 hour at room temperature.  
Abbreviations: ELISA (Cap) = ELISA capture, ICC = immunocytochemistry, IHC = immunohistochemistry, IP = immunoprecipitation, WB = western blot  
Hu = Human, Ms = Mouse, Rt = Rat, Ck = Chicken, F = Frog, B = Bovine

**Specificity**

The antibody detects a 140 kDa\* protein corresponding to eNOS on SDS-PAGE immunoblots of human umbilical vein endothelial cells.

\*All molecular weights (MW) are confirmed by comparison to MW standards and to western blot mobilities of known proteins with similar MW.  
"Native" western blot utilizes non-reducing sample buffer (no mercaptoethanol or SDS), normal SDS-PAGE gel electrophoresis, and no methanol in transfer buffers.

**Related Products**

NP2281 eNOS Rabbit Polyclonal

NM2321 eNOS (Ser-632), phospho-specific Mouse Monoclonal

NP2131 iNOS (C-terminal region) Rabbit Polyclonal

NP2141 nNOS (C-terminal region) Rabbit Polyclonal

NP4031 eNOS (Tyr-657)/nNOS (Tyr-895), phospho-specific Rabbit Polyclonal



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