

## Technical Data Sheet

**Purified Mouse Anti-Human eNOS (pS633)****Product Information**

<b>Material Number:</b>	612665
<b>Alternate Name:</b>	Nitric Oxide Synthase
<b>Size:</b>	150 µg
<b>Concentration:</b>	250 µg/ml
<b>Clone:</b>	37/eNOS(S633)
<b>Immunogen:</b>	Phosphorylated Human eNOS (pS633) Peptide
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human
<b>Target MW:</b>	140 kDa
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

**Description**

Nitric oxide synthase (NOS), a cell type specific enzyme, catalyzes the synthesis of nitric oxide (NO). NO is a short-lived radical that transmits signals involved in vasorelaxation, neurotransmission, and cytotoxicity. In neurons and endothelial cells, constitutive NOS (cNOS) is activated by agonists that increase intracellular Ca<sup>2+</sup> levels and enhance calmodulin binding. Neuronal NOS (nNOS) and endothelial NOS (eNOS) have recognition sites for NADPH, FAD, FMN, and calmodulin. eNOS has a unique N-myristylation consensus sequence that may explain its membrane localization. Various protein kinases have been implicated in regulation of eNOS activity, including AMPK, PKA, PKB/Akt, PKC, and CaM Kinase II. During VEGF stimulation, eNOS is transiently phosphorylated at Ser-1177 by PKB/Akt and dephosphorylated at Thr-495. At later time points, VEGF stimulation leads to an increase in Thr-495 phosphorylation mediated by PKC and a decrease in Ser-1177 phosphorylation. In addition, Ser-633 and Ser-1177 are phosphorylated by PKA and PKG in vitro. Thus, eNOS activity may be regulated through complex phosphorylation events mediated by multiple kinases at various phosphorylation sites.



**Western blot analysis for eNOS (pS633).** Human endothelial cells were either left untreated (lane 1) or were treated with 200 U/mL of lambda phosphatase for 1 hour at 37°C (lane 2). The top panel was probed with a mouse anti-eNOS antibody (Cat. No. 610296) and the bottom panel was probed with the mouse anti-eNOS (pS633) antibody at a 1:500 dilution with a band observable at ~ 140 kDa.

**Preparation and Storage**

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at -20°C.

**Application Notes****Application**

Western blot	Routinely Tested
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**Recommended Assay Procedure:**

**Western blot:** Please refer to [http://www.bdbiosciences.com/pharmingen/protocols/Western\\_Blotting.shtml](http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml)

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
611450	Human Endothelial Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
610296	Purified Mouse Anti-eNOS/NOS Type III	50 µg	3/eNOS/NOS Type III

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## References

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- Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation.. *Nature.* 1999; 399(6736):601-605.(Biology)
- Gallis B, Corthals GL, Goodlett DR, et al. Identification of flow-dependent endothelial nitric-oxide synthase phosphorylation sites by mass spectrometry and regulation of phosphorylation and nitric oxide production by the phosphatidylinositol 3-kinase inhibitor LY294002. *J Biol Chem.* 1999; 274(42):30101-30108. (Biology)
- Michell BJ, Chen Zp, Tiganis T, et al. Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *J Biol Chem.* 2001; 276(21):17625-17628.(Biology)