Technical Data Sheet

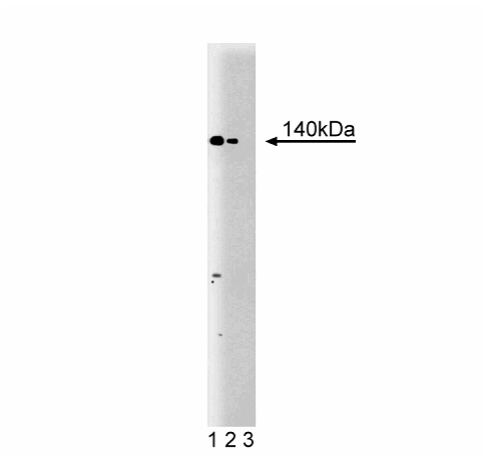
Polyclonal Rabbit Anti-eNOS/NOS Type III

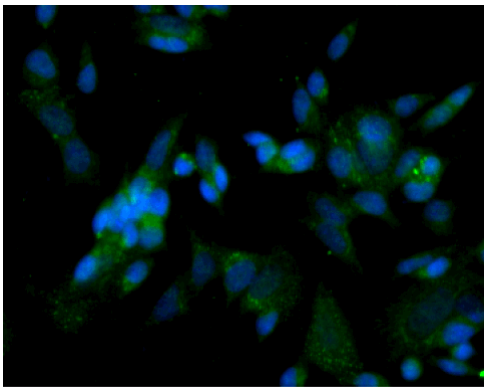
Product Information

Material Number:	**610298**
Alternate Name:	endothelial Nitric Oxide Synthase
Size:	50 µg
Concentration:	250 µg/ml
Clone:	Polyclonal
Immunogen:	Human eNOS aa. 1025-1203
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
Target MW:	140 kDa
Storage Buffer:	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[2+] levels and enhance calmodulin binding. Neuronal NOS (nNOS) and endothelial NOS (eNOS) have recognition sites for NADPH, FAD, FMN, and calmodulin and both are regulated in a similar manner. The human forms exhibit 52% amino acid identity. However, they are distinct gene products of about 155 kDa (nNOS) and 140 kDA (eNOS). The eNOS gene was cloned from human vascular endothelium as well as from bovine aortic endothelial cells (BAEC). eNOS protein has a unique N-myristylation consensus sequence that may explain its membrane localization.





Western blot analysis of eNOS/NOS Type III on a human endothelial lysate (left). *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the polyclonal rabbit anti-eNOS/NOS Type III antibody.*

Immunofluorescent staining of SH-SY5Y cells (Human neuroblastoma; ATCC CRL-2266) (right). *Cells were seeded in a collagen coated 384-well imaging plate (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) and the polyclonal rabbit anti-eNOS/NOS Type III antibody. The second step reagent used was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). The image was taken on a BD Pathway™ 855 or 435 imager using a 20x objective and merged with BD AttoVision™ software. This antibody also stained SK-N-SH (Human neuroblastoma; ATCC HTB-11), C6 (Rat glioma; ATCC CCL-107), U-87 MG (Human glioblastoma cells; ATCC HTB-14) and U-373 cells (Human glioblastoma cells; ATCC HTB-17; discontinued, investigators may refer to: http://www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx) using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

The polyclonal antibody was purified from antiserum by affinity chromatography.

Store undiluted at -20°C.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development

Recommended Assay Procedure:
Bioimaging:

Methanol Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611450	Human Endothelial Cell Lysate	500 µg	(none)
554021	HRP Goat Anti-Rabbit Ig	1.0 ml	(none)
353962	BD Falcon™ 384-well Imaging Plate	1 box	test clone

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/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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Chen PF, Tsai AL, Wu KK. Cysteine 184 of endothelial nitric oxide synthase is involved in heme coordination and catalytic activity. *J Biol Chem.* 1994; 269(40):25062-25066.(Biology)

Coers W, Timens W, Kempinga C, Klok PA, Moshage H. Specificity of antibodies to nitric oxide synthase isoforms in human, guinea pig, rat, and mouse tissues. *J Histochem Cytochem.* 1998; 46(12):1385-1391.(Biology: Immunohistochemistry, Immunoprecipitation, Western blot)

Ozaki M, Kawashima S, Yamashita T, et al. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J Clin Invest.* 2002; 110(3):331-340.(Biology: Western blot)

Sun J, Liao JK. Functional interaction of endothelial nitric oxide synthase with a voltage-dependent anion channel. *Proc Natl Acad Sci U S A.* 2002; 99(20):13108-13113.(Biology: Immunoprecipitation, Western blot)