Technical Data Sheet

Alexa Fluor® 647 Mouse anti-eNOS

Product Information

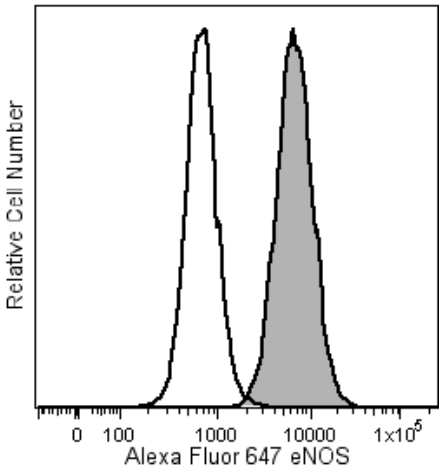
Material Number:	**560102**
Alternate Name:	NOS type III, NOS3, EC-NOS, NOS III
Size:	50 tests
Vol. per Test:	20 µl
Clone:	33/eNOS
Immunogen:	Human eNOS aa. 1025-1203
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed: Human Reported: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% 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 Ca2+ 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.

The 33/eNOS monoclonal antibody recognizes eNOS, regardless of phosphorylation status.

The specificity of this antibody conjugate for flow cytometric analysis was validated by confirming that RNA-mediated interference (RNAi) of the specific protein reduced the staining of the cells (see figure). Furthermore, the capacity of the RNAi to down-regulate the expression of the relevant protein was confirmed by western blot analysis.



Analysis of eNOS in human endothelial cells. *EA-hy 926 cells (Edgell, McDonald, Graham, 1983) were either transfected with eNOS RNAi (open histogram) or untreated (shaded histogram). The cells were fixed (BD Cytofix™ Fixation buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-eNOS. Down-regulation of eNOS expression is evident in the RNAi-transfected cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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Recommended Assay Procedure:
Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21
557870	Fix Buffer I	250 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. An isotype control should be used at the same concentration as the antibody of interest.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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. (Clone-specific: Western blot)

Dinerman JL, Dawson TM, Schell MJ, Snowman A, Snyder SH. Endothelial nitric oxide synthase localized to hippocampal pyramidal cells: implications for synaptic plasticity. *Proc Natl Acad Sci U S A.* 1994; 91(10):4214-4218. (Biology)

Edgell C-JS, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. *Proc Natl Acad Sci U S A.* 1983; 80:3734-3737. (Methodology: Controls)

Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. *J Biol Chem.* 1994; 269(19):13725-13728. (Biology)

Shen YH, Zhang L, Utama B et al. Human cytomegalovirus inhibits Akt-mediated eNOS activation through upregulating PTEN (phosphatase and tensin homolog deleted on chromosome 10). *Cardiovasc Res.* 2006; 69(2):502-511. (Biology)

Varghese P, Harrison RW, Lofthouse RA, Georgakopoulos D, Berkowitz DE, Hare JM. β3-adrenoceptor deficiency blocks nitric oxide-dependent inhibition of myocardial contractility. *J Clin Invest.* 2000; 106(5):697-703. (Clone-specific: Western blot)

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