

Anti-eNOS/NOS3 Antibody Picoband®

Catalog Number: A01604-3

About NOS3

NOS3 (Nitric Oxide Synthase 3), also called eNOS, an nitric oxide synthase that generates NO in blood vessels and is involved with regulating vascular tone by inhibiting smooth muscle contraction and platelet aggregation. The NOS3 gene is mapped on 7q36.1. Variations in this gene are associated with susceptibility to coronary spasm. Fulton et al. (1999) concluded the eNOS is an AKT substrate linking signal transduction by AKT to the release of the gaseous second messenger nitric oxide. AKT mediates the activation of eNOS, leading to increased nitric oxide production. Inhibition of the PI3K AKT pathway or mutation of the AKT site on eNOS protein at serine-1177 attenuated the serine phosphorylation and prevented the activation of eNOS. RT-PCR analysis showed that expression of NOS3 in human umbilical vein endothelial cells (HUVECs) and human aortic vascular smooth muscle cells (HAOSMCs) was inversely proportional to that of NOS3AS.

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Overview

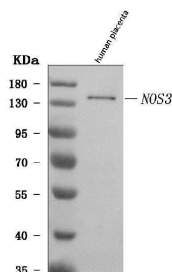
Product Name	Anti-eNOS/NOS3 Antibody Picoband®
Reactive Species	Human
Description	Boster Bio Anti-eNOS/NOS3 Antibody Picoband® catalog # A01604-3. Tested in ELISA, Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	P29474

Technical Details

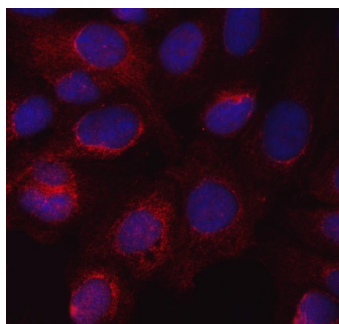
Immunogen	E.coli-derived human eNOS/NOS3 recombinant protein (Position: P34-Q1153).
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Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10⁶ cells, Human ELISA, 0.1-0.5 µg/ml, - Note: For flow cytometry (FCM), validated for fixed cells only. Not validated for FACS or surface flow cytometry using live cells.

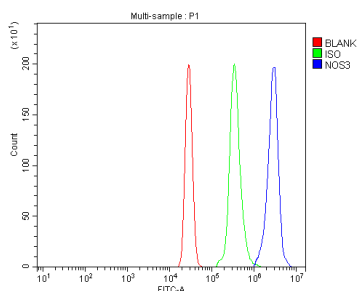
Anti-eNOS/NOS3 Antibody Picoband® (A01604-3) Images



Western blot analysis of eNOS/NOS3 using anti-eNOS/NOS3 antibody (A01604-3). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human placenta tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-eNOS/NOS3 antigen affinity purified polyclonal antibody (Catalog # A01604-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for eNOS/NOS3 at approximately 140 kDa. The expected band size for eNOS/NOS3 is at 133 kDa.



IF analysis of eNOS/NOS3 using anti-eNOS/NOS3 antibody (A01604-3). eNOS/NOS3 was detected in an immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-eNOS/NOS3 Antibody (A01604-3) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Flow Cytometry analysis of U937 cells using anti-eNOS/NOS3 antibody (A01604-3). Overlay histogram showing U937 cells stained with A01604-3 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-eNOS/NOS3 Antibody (A01604-3, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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