abcam

Product datasheet

Anti-eNOS (phospho S1177) antibody [EPR20991] - BSA and Azide free ab230158

Recombinant RabMAb

2 References 4 Images

Overview

Product name Anti-eNOS (phospho S1177) antibody [EPR20991] - BSA and Azide free

Description Rabbit monoclonal [EPR20991] to eNOS (phospho S1177) - BSA and Azide free

Host species Rabbit

Tested applications

Suitable for: WB, Dot blot, IP

Species reactivity

Reacts with: Mouse, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HUVEC +/- VEGF, whole cell lysate.

General notes ab230158 is the carrier-free version of ab215717.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR20991

Isotype IgG

Applications

Target

The Abpromise guarantee Our Abpromise guarantee covers the use of ab230158 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 140 kDa (predicted molecular weight: 133 kDa).
Dot blot		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Function	Produces nitric oxide (NO) which is implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway. NO mediates vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels and promotes blood clotting through the
	activation of platelets. Isoform eNOS13C: Lacks eNOS activity, dominant-negative form that may down-regulate eNOS activity by forming heterodimers with isoform 1.

Tissue specificity Platelets, placenta, liver and kidney.

Involvement in disease Variation in NOS3 seem to be associated with susceptibility to coronary spasm.

Sequence similarities Belongs to the NOS family.

Contains 1 FAD-binding FR-type domain.

Contains 1 flavodoxin-like domain.

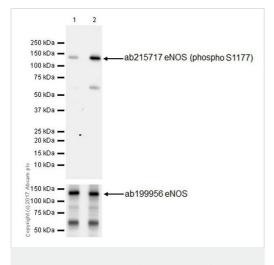
Post-translationalPhosphorylation by AMPK at Ser-1177 in the presence of Ca(2+)-calmodulin (CaM) activatesmodificationsactivity. In absence of Ca(2+)-calmodulin, AMPK also phosphorylates Thr-495, resulting ininhibition of activity (By similarity). Phosphorylation of Ser-114 by CDK5 reduces activity.

Cellular localization Cell membrane. Membrane, caveola. Cytoplasm, cytoskeleton. Golgi apparatus. Specifically

associates with actin cytoskeleton in the G2 phase of the cell cycle and which is favored by

interaction with NOSIP and results in a reduced enzymatic activity.

Images



Western blot - Anti-eNOS (phospho S1177) antibody [EPR20991] - BSA and Azide free (ab230158) **All lanes :** Anti-eNOS (phospho S1177) antibody [EPR20991] (ab215717) at 1/1000 dilution

Lane 1 : Untreated HUVEC (human umbilical vein endothelial cell line) whole cell lysate

Lane 2: HUVEC (human umbilical vein endothelial cell line) treated with 10 ng/ml VEGF for 5 min, whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 133 kDa Observed band size: 140 kDa

Exposure time: 3 minutes

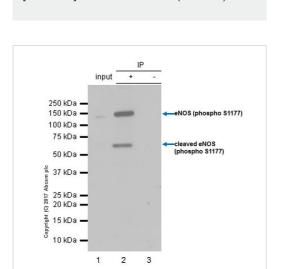
Blocking/Dilution buffer: 5% NFDM/TBST.

The 60 kDa band may be a cleaved form of eNOS (PMID: 16195740).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215717).



Dot Blot - Anti-eNOS (phospho S1177) antibody [EPR20991] - BSA and Azide free (ab230158)



Immunoprecipitation - Anti-eNOS (phospho S1177) antibody [EPR20991] - BSA and Azide free (ab230158)

Dot blot analysis of eNOS (phospho S1177) labeled with **ab215717** at 1/1000 dilution.

Lane 1: eNOS (phospho S1177) peptide.

Lane 2: eNOS non-phospho peptide.

Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Exposure time: 1 minute.

Blocking/Dilution buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215717).

eNOS (phospho S1177) was immunoprecipitated from 0.35 mg of HUVEC (human umbilical vein endothelial cell line) treated with 10 ng/ml VEGF for 5 min, whole cell lysate with <u>ab215717</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab215717</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: HUVEC treated with VEGF whole cell lysate 10 μg (Input). **Lane 2:** <u>ab215717</u> IP in HUVEC treated with VEGF whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of <u>ab215717</u> in HUVEC treated with VEGF whole cell lysate.

Exposure time: 30 seconds.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

The 60 kDa band may be a cleaved form of eNOS (PMID: 16195740).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and



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