SANTA CRUZ BIOTECHNOLOGY, INC.

p-NOS3 (pS633.37): sc-136198



BACKGROUND

Nitric oxide (NO), produced by the endothelial NO synthase (NOS3), is a fundamental determinant of cardiovascular homeostasis that maintains system blood pressure, vascular remodeling and angiogenesis. NOS3 is stimulated, in a phosphatidylinositol 3-kinase (PI 3-kinase)-dependent fashion, by treatment of endothelial cells with Insulin-like growth factor-1 and vascular endothelial growth factor (VEGF). The serine/threonine protein kinase Akt/PKB is an important downstream target of PI 3-kinase, regulating VEGF-stimulated endothelial cell survival. NOS3 activation via phosphorylation of Serine 1177 by Akt/PKB is necessary and sufficient for VEGF-mediated endothelial cell migration. Therefore, Akt/PKB can directly phosphorylate NOS3 on Serine 1177, activating the enzyme and leading to NO production.

REFERENCES

- Rudic, R.D., Shesely, E.G., Maeda, N., Smithies, O., Segal, S.S. and Sessa, W.C. 1998. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. J. Clin. Invest. 101: 731-736.
- Murohara, T., Asahara, T., Silver, M., Bauters, C., Masuda, H., Kalka, C., Kearney, M., Chen, D., Symes, J.F., Fishman, M.C., Huang, P.L. and Isner, J.M. 1998. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J. Clin. Invest. 101: 2567-2578.
- Chen, Z.P., Mitchelhill, K.I., Michell, B.J., Stapleton, D., Rodriguez-Crespo, I., Witters, L.A., Power, D.A., Ortiz de Montellano, P.R. and Kemp, B.E. 1999. AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett. 443: 285-289.
- Fulton, D., Gratton, J.P., McCabe, T.J., Fontana, J., Fujio, Y., Walsh, K., Franke, T.F., Papapetropoulos, A. and Sessa, W.C. 1999. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 399: 597-601.
- Dimmeler, S., Fleming, I., FissIthaler, B., Hermann, C., Busse, R. and Zeiher, A.M. 1999. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399: 601-605.
- Dimmeler, S., Dernbach, E. and Zeiher, A.M. 2000. Phosphorylation of the endothelial nitric oxide synthase at Ser-1177 is required for VEGF-induced endothelial cell migration. FEBS Lett. 477: 258-262.

CHROMOSOMAL LOCATION

Genetic locus: NOS3 (human) mapping to 7q36.1.

SOURCE

p-NOS3 (pS633.37) is a mouse monoclonal antibody raised against a short amino acid sequence containing Ser 633 phosphorylated NOS3 of human origin.

PRODUCT

Each vial contains 200 μg lgG_1 kappa light chain in 1.0 ml of PBS with <0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

p-NOS3 (pS633.37) is recommended for detection of Ser 633 phosphorylated NOS3 of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)].

Suitable for use as control antibody for NOS3 siRNA (h): sc-36093, NOS3 shRNA Plasmid (h): sc-36093-SH and NOS3 shRNA (h) Lentiviral Particles: sc-36093-V.

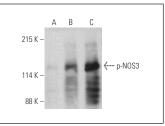
Molecular Weight of p-NOS3: 140 kDa.

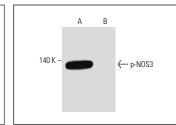
Positive Controls: HUV-EC-C whole cell lysate: sc-364180.

RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGĸ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

DATA





p-NOS3 (pS633.37): sc-136198. Western blot analysis of NOS3 (pS633.37): sc-136198. Western blot analysis of NOS3 phosphorylation in untreated (**A**), VEGF-treated (**B**) and Forskolin-treated (**C**) HUV-EC-C whole cell lysates. Blocked with UltraCruz[®] Blocking Reagent: sc-516124. Detection reagent used: m-IqGk: BP-HRP: sc-516102.

p-NOS3 (pS633.37): sc-136198. Western blot analysis of NOS3 phosphorylation in human endothelial cells either untreated $({\bf A})$ or treated $({\bf B})$ with lambda phosphatase.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures. Not for resale.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.