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SZABO-SCANDIC Handels GmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

ENOPH1 siRNA (m): sc-144654

BACKGROUND

ENOPH1 (enolase-phosphatase 1), also known as E1, MASA or MST145, is a member of the MasA family of the HAD (halo-acid dehalogenase)-like hydrolase superfamily. Existing as a monomer and binding magnesium as a cofactor, ENOPH1 is a bifunctional enzyme, exhibiting both phosphatase and atypical enolase activities. ENOPH1 plays an important role in the ubiquitous methionine salvage pathway, a biochemical pathway found in all organisms that regulates methionine levels in the cell (also known as the Yang cycle in plants). More specifically, ENOPH1 catalyzes the continuous enolization and dephosphorylation of 2,3-diketo-5-methylthio-1-phosphopentane to yield the acireductone metabolite 1,2-dihydroxy-3-keto-5-methylthiopentene. Due to alternative splicing events, two isoforms exist for ENOPH1.

REFERENCES

1. Zhang, Y., et al. 2004. Analogs of 1-phosphonoxy-2,2-dihydroxy-3-oxo-5-(methylthio)pentane, an acyclic intermediate in the methionine salvage pathway: a new preparation and characterization of activity with E1 enolase/phosphatase from *Klebsiella oxytoca*. *Bioorg. Med. Chem.* 12: 3847-3855.
2. Kostic, M., et al. 2004. (1)H, (13)C and (15)N chemical shift assignments of an enolase-phosphatase, E1, from *Klebsiella oxytoca*. *J. Biomol. NMR* 30: 359-360.
3. Wang, H., et al. 2005. Purification, crystallization and preliminary X-ray diffraction analysis of human enolase-phosphatase E1. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 61: 521-523.

CHROMOSOMAL LOCATION

Genetic locus: Enoph1 (mouse) mapping to 5 E4.

PRODUCT

ENOPH1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ENOPH1 shRNA Plasmid (m): sc-144654-SH and ENOPH1 shRNA (m) Lentiviral Particles: sc-144654-V as alternate gene silencing products.

For independent verification of ENOPH1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-144654A, sc-144654B and sc-144654C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ENOPH1 siRNA (m) is recommended for the inhibition of ENOPH1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

ENOPH1 (H-10): sc-365155 is recommended as a control antibody for monitoring of ENOPH1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ENOPH1 gene expression knockdown using RT-PCR Primer: ENOPH1 (m)-PR: sc-144654-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Mutze, K., et al. 2015. Enolase 1 (ENO1) and protein disulfide-isomerase associated 3 (PDIA3) regulate Wnt/ β -catenin-driven *trans*-differentiation of murine alveolar epithelial cells. *Dis. Model. Mech.* 8: 877-890.
2. Zhang, Y., et al. 2016. Cerebral microvascular endothelial cell apoptosis after ischemia: role of enolase-phosphatase 1 activation and acireductone dioxygenase 1 translocation. *Front. Mol. Neurosci.* 9: 79.

RESEARCH USE

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

PROTOCOLS

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