

MetAP-1 siRNA (h): sc-61022

BACKGROUND

Methionine aminopeptidases (MetAP), also designated peptidase M proteins, are members of the M24 family of proteins. MetAP proteins remove the amino-terminal methionine residue from nascent polypeptides. MetAP-1 is a 394-amino acid protein that is expressed at low levels in all tissues, but is highly expressed in skeletal muscles. The active site of MetAP-1 contains two adjacent divalent metal ions connected by a water molecule or hydroxide ion. The control of cell proliferation in mammalian cells is directly linked and strictly dependent on the evolutionarily highly conserved mechanism that MetAP-1 employs. Eukaryotes contain both MetAP-1 and MetAP-2, whereas prokaryotes possess only the MetAP-1 enzyme. Pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT) forms a scaffold that inhibits the action of MetAP-1, while 1,2,4-triazol is a non-peptide inhibitor of MetAP-1 binding to the active site with the N1 and N2 atoms of the triazole moiety complexing two divalent ions.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610151. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Oefner, C., et al. 2003. The 1.15Å crystal structure of the *Staphylococcus aureus* methionyl-aminopeptidase and complexes with triazole based inhibitors. J. Mol. Biol. 332: 13-21.
3. Brdlik, C.M. and Crews, C.M. 2004. A single amino acid residue defines the difference in ovalicin sensitivity between type I and II methionine aminopeptidases. J. Biol. Chem. 279: 9475-9480.
4. Swierczek, K., et al. 2005. Molecular discrimination of type-I over type-II methionyl aminopeptidases. Biochemistry 44: 12049-12056.
5. Bernier, S.G., et al. 2005. Methionine aminopeptidases type I and type II are essential to control cell proliferation. J. Cell. Biochem. 95: 1191-1203.
6. Luo, Q.L., et al. 2005. Inhibitors of type I MetAPs containing pyridine-2-carboxylic acid thiazol-2-ylamide. Part 1: SAR studies on the determination of the key scaffold. Bioorg. Med. Chem. Lett. 15: 635-638.

CHROMOSOMAL LOCATION

Genetic locus: METAP1 (human) mapping to 4q23.

PRODUCT

MetAP-1 siRNA (h) 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 MetAP-1 shRNA Plasmid (h): sc-61022-SH and MetAP-1 shRNA (h) Lentiviral Particles: sc-61022-V as alternate gene silencing products.

For independent verification of MetAP-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61022A, sc-61022B and sc-61022C.

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

MetAP-1 siRNA (h) is recommended for the inhibition of MetAP-1 expression in human 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

MetAP-1 (A-2): sc-514653 is recommended as a control antibody for monitoring of MetAP-1 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 MetAP-1 gene expression knockdown using RT-PCR Primer: MetAP-1 (h)-PR: sc-61022-PR (20 μ l, 600 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.