

VHY siRNA (m): sc-61787

BACKGROUND

Mitogen-activated protein (MAP) kinases are a large class of proteins involved in signal transduction pathways that are activated by a range of stimuli and mediate a number of physiological and pathological changes in the cell. Dual specificity phosphatases (DSPs) are a subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which are selective for dephosphorylating critical phosphothreonine and phosphotyrosine residues within MAP kinases. DSP gene expression is induced by a host of growth factors and/or cellular stresses, thereby negatively regulating MAP kinase superfamily members including MAPK/ERK, SAPK/JNK and p38. VHY-related member Y (VHY) is a member of a subgroup of myristoylated VH1-like small dual specificity phosphatases. It is highly expressed in testis, specifically in pachytene spermatocytes (midstage of meiotic division I) and round spermatids. VHY localizes to the plasma membrane in transfected 293T or NIH/3T3 cells.

REFERENCES

1. Keyse, S.M. 1995. An emerging family of dual specificity MAP kinase phosphatases. *Biochem. Biophys. Acta* 1265: 152-160.
2. Sun, H. 1998. Functional studies of dual-specificity phosphatases. *Methods Mol. Biol.* 84: 307-318.
3. Camps, M., et al. 2000. Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J.* 14: 6-16.
4. Alonso, A., et al. 2004. VHY, a novel myristoylated testis-restricted dual specificity protein phosphatase related to VHX. *J. Biol. Chem.* 279: 32586-32591.
5. Alonso, A., et al. 2004. The minimal essential core of a cysteine-based protein-tyrosine phosphatase revealed by a novel 16-kDa VH1-like phosphatase, VHZ. *J. Biol. Chem.* 279: 35768-35774.
6. Yoon, T.S., et al. 2005. Crys protein phosphatase. *Proteins* 61: 694-697.

CHROMOSOMAL LOCATION

Genetic locus: Dusp15 (mouse) mapping to 2 H1.

PRODUCT

VHY 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 VHY shRNA Plasmid (m): sc-61787-SH and VHY shRNA (m) Lentiviral Particles: sc-61787-V as alternate gene silencing products.

For independent verification of VHY (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-61787A, sc-61787B and sc-61787C.

PROTOCOLS

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

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

VHY siRNA (m) is recommended for the inhibition of VHY 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

VHY (G-7): sc-166173 is recommended as a control antibody for monitoring of VHY 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 VHY gene expression knockdown using RT-PCR Primer: VHY (m)-PR: sc-61787-PR (20 μ l). 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.