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SANTA CRUZ BIOTECHNOLOGY, INC.

PLEKHA4 siRNA (m): sc-152305

BACKGROUND

PLEKHA4 (pleckstrin homology domain-containing family A member 4), also known as PEPP1 (phosphoinositol 3-phosphate-binding protein 1), is a 779 amino acid protein that contains one Pleckstrin homology (PH) domain, which is found in proteins that are involved in intracellular signaling. PLEKH4A specifically binds to PtdIns3P (phosphatidylinositol-3-phosphate), a phospholipid that resides on early endosomes, but not to other phosphoinositides. Though detected at low levels in normal skeletal muscle, small intestine, liver, heart and kidney, PLEKHA4 is found to be highly expressed in melanoma. The gene encoding PLEKH4 maps to human chromosome 19, which consists of over 63 million bases, houses approximately 1,400 genes and is recognized for having the greatest gene density of the human chromosomes. There are two isoforms of PLEKHA4 that are produced as a result of alternative splicing events.

REFERENCES

1. Gilbert, F. 1997. Disease genes and chromosomes: disease maps of the human genome. Chromosome 19. Genet. Test. 1: 145-149.
2. Dowler, S., et al. 2000. Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. Biochem. J. 351: 19-31.
3. Lemmon, M.A. and Ferguson, K.M. 2001. Molecular determinants in pleckstrin homology domains that allow specific recognition of phosphoinositides. Biochem. Soc. Trans. 29: 377-384.
4. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 607769. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Lemmon, M.A. 2004. Pleckstrin homology domains: not just for phosphoinositides. Biochem. Soc. Trans. 32: 707-711.
6. Cozier, G.E., et al. 2004. Membrane targeting by pleckstrin homology domains. Curr. Top. Microbiol. Immunol. 282: 49-88.
7. Grimwood, J., et al. 2004. The DNA sequence and biology of human chromosome 19. Nature 428: 529-535.
8. Subramanian, D., et al. 2010. Activation of membrane-permeant caged PtdIns(3)P induces endosomal fusion in cells. Nat. Chem. Biol. 6: 324-326.

CHROMOSOMAL LOCATION

Genetic locus: Plekha4 (mouse) mapping to 7 B4.

PRODUCT

PLEKHA4 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PLEKHA4 shRNA Plasmid (m): sc-152305-SH and PLEKHA4 shRNA (m) Lentiviral Particles: sc-152305-V as alternate gene silencing products.

For independent verification of PLEKHA4 (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-152305A, sc-152305B and sc-152305C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PLEKHA4 siRNA (m) is recommended for the inhibition of PLEKHA4 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 µM in 66 µl. 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

PLEKHA4 (H-1): sc-376408 is recommended as a control antibody for monitoring of PLEKHA4 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-IgGκ 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, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PLEKHA4 gene expression knockdown using RT-PCR Primer: PLEKHA4 (m)-PR: sc-152305-PR (20 µl). 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.