

PHLDA3 siRNA (m): sc-152226

BACKGROUND

PHLDA3 (pleckstrin homology-like domain, family A, member 3), also known as TIH1, is a 127 amino acid protein that contains one PH domain and is expressed in a variety of tissues, with particularly low expression in spleen and liver tissue. Sharing nearly 100% sequence homology with its mouse counterpart, PHLDA3 functions as a tumor suppressor that inhibits Akt signaling and is required for p53-dependent apoptosis. The gene encoding PHLDA3 maps to human chromosome 1, which spans 260 million base pairs, contains over 3,000 genes and comprises nearly 8% of the human genome. Chromosome 1 houses a large number of disease-associated genes, including those that are involved in familial adenomatous polyposis, Stickler syndrome, Parkinson's disease, Gaucher disease, schizophrenia and Usher syndrome. Aberrations in chromosome 1 are found in a variety of cancers, including head and neck cancer, malignant melanoma and multiple myeloma.

REFERENCES

1. Maruyama, K. and Sugano, S. 1994. Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides. *Gene* 138: 171-174.
2. Frank, D., et al. 1999. A novel pleckstrin homology-related gene family defined by Ipl/Tssc3, TDAG51, and Tih1: tissue-specific expression, chromosomal location, and parental imprinting. *Mamm. Genome* 10: 1150-1159.
3. Gottlieb, T.M., et al. 2002. Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. *Oncogene* 21: 1299-1303.
4. Kawase, T., et al. 2009. PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt. *Cell* 136: 535-550.

CHROMOSOMAL LOCATION

Genetic locus: Phlda3 (mouse) mapping to 1 E4.

PRODUCT

PHLDA3 siRNA (m) is a pool of 2 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 PHLDA3 shRNA Plasmid (m): sc-152226-SH and PHLDA3 shRNA (m) Lentiviral Particles: sc-152226-V as alternate gene silencing products.

For independent verification of PHLDA3 (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-152226A and sc-152226B.

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

PHLDA3 siRNA (m) is recommended for the inhibition of PHLDA3 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

PHLDA3 (A-10): sc-518201 is recommended as a control antibody for monitoring of PHLDA3 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 PHLDA3 gene expression knockdown using RT-PCR Primer: PHLDA3 (m)-PR: sc-152226-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.

PROTOCOLS

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