

PHIP siRNA (h): sc-62800

BACKGROUND

PHIP (pleckstrin homology domain interacting protein), also known as ndrp or WDR11, is a 1,821 amino acid protein that contains 8 N-terminal WD40 repeats and 2 bromodomains. It is expressed in skeletal muscle (localizing to the cytosol and nucleus) and primary β cells (localizing to the nucleus) and acts as a transcriptional activator. PHIP is known to interact with various members of the Insulin receptor substrate (IRS) family. The IRS family of proteins mediate Insulin receptor signaling and play an important role in Insulin-producing β cell proliferation and survival. PHIP specifically associates with the PH domain of IRS-1 and may function to link IRS-1 to Insulin receptors, indicating a vital role of PHIP in the regulation of Insulin signaling. Further supporting this role of PHIP, mutations in the gene encoding PHIP disrupt IRS-mediated signaling pathways resulting in the inhibition of GLUT4 translocation in muscle cells. PHIP is also known to bind IRS-2 and may play a similar role; linking IRS-2 to Insulin receptors.

REFERENCES

1. Farhang-Fallah, J., et al. 2000. Cloning and characterization of PHIP, a novel Insulin receptor substrate-1 pleckstrin homology domain interacting protein. *J. Biol. Chem.* 275: 40492-40497.
2. Farhang-Fallah, J., et al. 2002. The pleckstrin homology (PH) domain-interacting protein couples the Insulin receptor substrate 1 PH domain to Insulin signaling pathways leading to mitogenesis and GLUT4 translocation. *Mol. Cell. Biol.* 22: 7325-7336.
3. Sadagurski, M., et al. 2005. Insulin receptor substrate 2 plays diverse cell-specific roles in the regulation of glucose transport. *J. Biol. Chem.* 280: 14536-14544.
4. Podcheko, A., et al. 2007. Identification of a WD40 repeat-containing isoform of PHIP as a novel regulator of β -cell growth and survival. *Mol. Cell. Biol.* 27: 6484-6496.
5. Kaburagi, Y., et al. 2007. Role of IRS and PHIP on Insulin-induced tyrosine phosphorylation and distribution of IRS proteins. *Cell Struct. Funct.* 32: 69-78.
6. Hosooka, T., et al. 2008. Dok1 mediates high-fat diet-induced adipocyte hypertrophy and obesity through modulation of PPAR- γ phosphorylation. *Nat. Med.* 14: 188-193.

CHROMOSOMAL LOCATION

Genetic locus: PHIP (human) mapping to 6q14.1.

PRODUCT

PHIP 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 PHIP shRNA Plasmid (h): sc-62800-SH and PHIP shRNA (h) Lentiviral Particles: sc-62800-V as alternate gene silencing products.

For independent verification of PHIP (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-62800A, sc-62800B and sc-62800C.

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

PHIP siRNA (h) is recommended for the inhibition of PHIP 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

PHIP (B-1): sc-398614 is recommended as a control antibody for monitoring of PHIP 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 PHIP gene expression knockdown using RT-PCR Primer: PHIP (h)-PR: sc-62800-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.