

PRR5 siRNA (h): sc-76261

BACKGROUND

Proline-rich proteins are often involved in protein-protein interactions and typically act as ligands for SH3, WW and EVH1 domains. PRR5 (proline-rich protein 5), also known as protein observed with Rictor-1 (Protor-1), is a 388 amino acid protein that is a subunit of mammalian target of Rapamycin complex 2 (mTORC2), a multimeric kinase that is involved in the regulation of the Actin cytoskeleton and phosphorylates Akt1, SGK and PKC α . Other proteins that make up mTORC2 include Rictor, MAPKAP-1, FRAP and LST8. PRR5 plays an important role in the modulation of platelet-derived growth factor signaling and in the regulation of PDGFR- β expression. Since the gene encoding PRR5 is located in a region that frequently undergoes loss of heterozygosity in breast cancer, it is likely that PRR5 is a tumor suppressor. There are four isoforms of PRR5 that are produced as a result of alternative splicing events.

REFERENCES

1. Johnstone, C.N., et al. 2005. PRR5 encodes a conserved proline-rich protein predominant in kidney: analysis of genomic organization, expression, and mutation status in breast and colorectal carcinomas. *Genomics* 85: 338-351.
2. Pearce, L.R., et al. 2007. Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem. J.* 405: 513-522.
3. Woo, S.Y., et al. 2007. PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor β expression and signaling. *J. Biol. Chem.* 282: 25604-25612.
4. Thedieck, K., et al. 2007. PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS ONE* 2: e1217.
5. Nakamichi, N., et al. 2007. *Arabidopsis* clock-associated pseudo-response regulators PRR9, PRR7 and PRR5 coordinately and positively regulate flowering time through the canonical CONSTANS-dependent photoperiodic pathway. *Plant Cell Physiol.* 48: 822-832.

CHROMOSOMAL LOCATION

Genetic locus: PRR5 (human) mapping to 22q13.31.

PRODUCT

PRR5 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 PRR5 shRNA Plasmid (h): sc-76261-SH and PRR5 shRNA (h) Lentiviral Particles: sc-76261-V as alternate gene silencing products.

For independent verification of PRR5 (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-76261A, sc-76261B and sc-76261C.

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

PRR5 siRNA (h) is recommended for the inhibition of PRR5 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

PRR5 (A-7): sc-390469 is recommended as a control antibody for monitoring of PRR5 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 PRR5 gene expression knockdown using RT-PCR Primer: PRR5 (h)-PR: sc-76261-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.