

RIP5 siRNA (h): sc-88815

BACKGROUND

The phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions in eukaryotes, including cell division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the serine/threonine (Ser/Thr) protein kinases. RIP5 (receptor interacting protein kinase 5), also known as RPK5, DustyPK or SGK496 (sugen kinase 496), is a 929 amino acid protein that localizes to the cytoplasm, contains one protein kinase domain and belongs to the Ser/Thr protein kinase family. Expressed at low levels in placenta, heart, brain, kidney, pancreas, testis and skeletal muscle, RIP5 catalyzes the ATP-dependent phosphorylation of target proteins and is thought to induce both caspase-dependent and -independent cell death. Four isoforms of RIP5 exist due to alternative splicing events.

REFERENCES

1. Bairoch, A., et al. 1988. Sequence patterns in protein kinases. *Nature* 331: 22.
2. Hanks, S.K., et al. 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241: 42-52.
3. Hunter, T. 1991. Protein kinase classification. *Methods Enzymol.* 200: 3-37.
4. Hanks, S.K., et al. 1991. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol.* 200: 38-62.
5. Zha, J., et al. 2004. RIP5 is a RIP-homologous inducer of cell death. *Biochem. Biophys. Res. Commun.* 319: 298-303.
6. Peng, J., et al. 2006. Dusty protein kinases: primary structure, gene evolution, tissue specific expression and unique features of the catalytic domain. *Biochim. Biophys. Acta* 1759: 562-572.

CHROMOSOMAL LOCATION

Genetic locus: DSTYK (human) mapping to 1q32.1.

PRODUCT

RIP5 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 RIP5 shRNA Plasmid (h): sc-88815-SH and RIP5 shRNA (h) Lentiviral Particles: sc-88815-V as alternate gene silencing products.

For independent verification of RIP5 (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-88815A, sc-88815B and sc-88815C.

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.

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

RIP5 siRNA (h) is recommended for the inhibition of RIP5 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

RIP5 (E-6): sc-374487 is recommended as a control antibody for monitoring of RIP5 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 RIP5 gene expression knockdown using RT-PCR Primer: RIP5 (h)-PR: sc-88815-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.