

# RIP4 siRNA (h): sc-91500

## 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. RIP4, also known as RPK4 (receptor-interacting serine/threonine-protein kinase 4), PKK (PKC  $\delta$ -interacting protein kinase), DIK, ANKK2 or ANKRD3 (Ankyrin repeat domain-containing protein 3), is a peripheral membrane protein that belongs to the TKL Ser/Thr protein kinase family and functions as a receptor-interacting protein (RIP) that modulates epidermal growth and differentiation. RIPs interact with the intracellular domain of tumor necrosis factor receptors (TNFRs) and facilitate downstream signaling and apoptosis induction. RIP4 contains ten Ankyrin-repeats and one protein kinase domain, and participates in the activation of NF $\kappa$ B. Two isoforms exist due to alternative splicing events.

## REFERENCES

1. Bhr, C., et al. 2000. DIK, a novel protein kinase that interacts with protein kinase C  $\delta$ . Cloning, characterization, and gene analysis. *J. Biol. Chem.* 275: 36350-36357.
2. Holland, P., et al. 2002. RIP4 is an Ankyrin repeat-containing kinase essential for keratinocyte differentiation. *Curr. Biol.* 12: 1424-1428.
3. Meylan, E., et al. 2002. RIP4 (DIK/PKK), a novel member of the RIP kinase family, activates NF $\kappa$ B and is processed during apoptosis. *EMBO Rep.* 3: 1201-1208.

## CHROMOSOMAL LOCATION

Genetic locus: RPK4 (human) mapping to 21q22.3.

## PRODUCT

RIP4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RIP4 shRNA Plasmid (h): sc-91500-SH and RIP4 shRNA (h) Lentiviral Particles: sc-91500-V as alternate gene silencing products.

For independent verification of RIP4 (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-91500A, sc-91500B and sc-91500C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

RIP4 siRNA (h) is recommended for the inhibition of RIP4 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  $\mu$ M in 66  $\mu$ 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

RIP4 (E-7): sc-377368 is recommended as a control antibody for monitoring of RIP4 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RIP4 gene expression knockdown using RT-PCR Primer: RIP4 (h)-PR: sc-91500-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Yi, Z., et al. 2020. Silencing of RPK4 inhibits epithelial-mesenchymal transition by inactivating the Wnt/ $\beta$ -catenin signaling pathway in osteosarcoma. *Mol. Med. Rep.* 21: 1154-1162.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.