SANTA CRUZ BIOTECHNOLOGY, INC.

RICK siRNA (h): sc-37389

BACKGROUND

Members of the tumor necrosis factor receptor (TNFR) family play a key role in the induction of NFκB activation and cell death. These receptors recruit and assemble signaling complexes that contain a number of death-domain containing proteins, such as RIP. RICK, also designated RIP2 and CARDIAK, is a RIP-like protein kinase involved in regulating both TNFR and CD95-mediated apoptosis. RICK contains an N-terminal serine-threonine kinase catalytic domain and a C-terminal caspase-recruiting domain. The C-terminal domain is sufficient for the apoptotic functions of the protein, while the whole protein is required for the activation of NFκB. RICK binds specifically to a number of proteins in the TNFR-associated factor (TRAF) family, and these TRAF interactions are involved in recruiting RICK to receptor signaling complexes. Overexpression of RICK leads to the activation of caspase-8 and potentiates apoptosis induced by Fas ligand, FADD, CLARP and caspase-8.

REFERENCES

1. Ware, C.F., et al. 1996. Apoptois mediated by the TNF-related cytokine and receptor families. J. Cell. Biochem. 60: 47-55.
2. Marsters, S.A., et al. 1996. Apo-3, a new member of the tumor necrosis factor receptor family contains a death domain and activates apoptosis and NFκB. Curr. Biol. 6: 1669-1676.
3. Lee, S.Y., et al. 1997. TRAF2 is essential for JNK but not NFκB activation and regulates lymphocyte proliferation and survival. Immunity 7: 703-713.
4. Thome, M., et al. 1998. Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. Curr. Biol. 8: 885-888.
5. Inohara, N., et al. 1998. RICK, a novel protein kinase containing a caspase recruitment domain, interacts with CLARP and regulates CD95-mediated apoptosis. J. Biol. Chem. 273: 12296-12300.
6. McCarthy, J.V., et al. 1998. RIP2 is a novel NFκB-activating and cell death-inducing kinase. J. Biol. Chem. 273: 16968-16975.
7. Kobayashi, K. et al. 2002. RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416: 194-199.

CHROMOSOMAL LOCATION

Genetic locus: RIPK2 (human) mapping to 8q21.3.

PRODUCT

RICK 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RICK shRNA Plasmid (h): sc-37389-SH and RICK shRNA (h) Lentiviral Particles: sc-37389-V as alternate gene silencing products.

For independent verification of RICK (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-37389A, sc-37389B and sc-37389C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RICK siRNA (h) is recommended for the inhibition of RICK 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 µM in 66 µl. 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

RICK (A-10): sc-166765 is recommended as a control antibody for monitoring of RICK 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RICK gene expression knockdown using RT-PCR Primer: RICK (h)-PR: sc-37389-PR (20 µl, 481 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Fukazawa, A., et al. 2008. GEF-H1 mediated control of NOD1 dependent NFκB activation by *Shigella* effectors. PLoS Pathog. 4: e1000228.

RESEARCH USE

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