SANTA CRUZ BIOTECHNOLOGY, INC.

RBCK1 siRNA (h): sc-61446

BACKGROUND

The RING finger motif is a specialized DNA-binding zinc finger domain found in many transcriptional regulatory proteins. RBCC protein interacting with PKC 1 (RBCK1), a member of the RING-IBR protein family, interacts with β-I-type (PRKCB1) and Z-type protein kinase C (PRKCZ) as well as UBE2L3, and has a new type of RING-B-Box-Coiled-Coil (RBCC) region. RBCK1 can form homodimers *in vitro* and is a transcription factor with both transcriptional and DNA-binding activities that are unlike other RBCC family proteins. RBCK1 shuttles between the cytoplasm and nucleus and possesses nuclear export and localization signals within its amino acid sequence. It may function as an E3 ubiquitin-protein ligase, or as a part of the E3 complex, which accepts ubiquitin from E2 ubiquitin-conjugating enzymes, such as UBE2L3/UBCM4, and then transfers ubiquitin to substrates.

REFERENCES

1. Tokunaga, C., et al. 1998. Molecular cloning and characterization of a novel protein kinase C-interacting protein with structural motifs related to RBCC family proteins. Biochem. Biophys. Res. Commun. 244: 353-359.
2. Tatematsu, K., et al. 1998. Transcriptional activity of RBCK1 protein (RBCC protein interacting with PKC 1): requirement of RING-finger and B-box motifs and regulation by protein kinases. Biochem. Biophys. Res. Commun. 247: 392-396.
3. Tokunaga, C., et al. 1998. Molecular cloning and characterization of RBCK2, a splicing variant of a RBCC family protein, RBCK1. FEBS Lett. 435: 11-15.
4. Tatematsu, K., et al. 2005. Nuclear-cytoplasmic shuttling of a RING-IBR protein RBCK1 and its functional interaction with nuclear body proteins. J. Biol. Chem. 280: 22937-22944.

CHROMOSOMAL LOCATION

Genetic locus: RBCK1 (human) mapping to 20p13.

PRODUCT

RBCK1 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 RBCK1 shRNA Plasmid (h): sc-61446-SH and RBCK1 shRNA (h) Lentiviral Particles: sc-61446-V as alternate gene silencing products.

For independent verification of RBCK1 (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-61446A, sc-61446B and sc-61446C.

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

RBCK1 siRNA (h) is recommended for the inhibition of RBCK1 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

RBCK1 (E-2): sc-365523 is recommended as a control antibody for monitoring of RBCK1 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 RBCK1 gene expression knockdown using RT-PCR Primer: RBCK1 (h)-PR: sc-61446-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Brazee, P.L., et al. 2019. Linear ubiquitin assembly complex regulates lung epithelial driven responses during influenza infection. J. Clin. Invest. 12 pii: 128368.

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.