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SANTA CRUZ BIOTECHNOLOGY, INC.

CNK1 siRNA (m): sc-142433

BACKGROUND

CNK1 (connector enhancer of kinase suppressor of Ras 1), also known as KSR or CNKSR1, is a 720 amino acid cytoplasmic and membrane protein involved in the RAS-dependent signaling pathway. RAS is essential for integrating and transmitting proliferation, differentiation and survival signals elicited by membrane receptors to downstream effector pathways. RAF is part of the RAS dependent signaling pathway and is involved in the transduction of mitogenic signals from the cell membrane to the nucleus. CNK1 contains several protein-protein interaction domains and plays a key role as a tyrosine phosphorylation target in multiple receptor tyrosine kinase pathways. The C-terminal portion of CNK1 directly binds to RAF, blocking RAS- and RAF-dependent signaling when overexpressed. The N-terminal portion contains two domains that are critical for cooperation with RAS, and two CNK1 isoforms are produced due to alternative splicing events.

REFERENCES

1. Ishikawa, F., et al. 1987. Rat c-raf oncogene activation by a rearrangement that produces a fused protein. Mol. Cell. Biol. 7: 1226-1232.
2. Katz, M.E., et al. 1997. Signal transduction from multiple Ras effectors. Curr. Opin. Genet. Dev. 7: 75-79.
3. Therrien, M., et al. 1998. CNK, a RAF-binding multidomain protein required for RAS signaling. Cell 95: 343-353.
4. Bos, J.L. 1998. All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. EMBO J. 17: 6776-6782.
5. Therrien, M., et al. 1999. Functional analysis of CNK in RAS signaling. Proc. Natl. Acad. Sci. USA 96: 13259-13263.
6. Jaffe, A.B., et al. 2004. Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. Mol. Cell. Biol. 24: 1736-1746.
7. Fritz, R.D., et al. 2005. The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor. Biochem. Biophys. Res. Commun. 338: 1906-1912.

CHROMOSOMAL LOCATION

Genetic locus: Cnksr1 (mouse) mapping to 4 D3.

PRODUCT

CNK1 siRNA (m) 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 CNK1 shRNA Plasmid (m): sc-142433-SH and CNK1 shRNA (m) Lentiviral Particles: sc-142433-V as alternate gene silencing products.

For independent verification of CNK1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142433A, sc-142433B and sc-142433C.

RESEARCH USE

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

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

CNK1 siRNA (m) is recommended for the inhibition of CNK1 expression in mouse 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

CNK1 (G-7): sc-514607 is recommended as a control antibody for monitoring of CNK1 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 CNK1 gene expression knockdown using RT-PCR Primer: CNK1 (m)-PR: sc-142433-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.