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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

CrkRS shRNA (m) Lentiviral Particles: sc-44531-V

BACKGROUND

CrkRS (Cdc2-related kinase, arginine/serine-rich, also designated CRK7 and CRKR) is an ubiquitous protein that appears to localize to the nucleus and link transcription and splicing machinery. CrkRS belongs to the serine/threonine protein kinase family and Cdc2/Cdkx subfamily. CrkRS has extensive proline-rich regions that resemble SH3 and WW domain binding sites, and an RS domain that is characteristic of splicing factors. The protein kinase domain of CrkRS is 89% identical to the CHED protein kinase, also designated CDC2L5 and cell division cycle 2-like 5 (cholinesterase-related cell division controller), however outside the kinase domains the two proteins are unique. Cell cycle control kinases can phosphorylate proteins important for differentiation and apoptosis and provide connections between proliferation, differentiation, apoptosis, and neurocytoskeleton dynamics.

REFERENCES

1. Lapidot-Lifson, Y., et al. 1992. Cloning and antisense oligodeoxynucleotide inhibition of a human homolog of cdc2 required in hematopoiesis. *Proc. Natl. Acad. Sci. USA* 89: 579-583.
2. Meyerson, M., et al. 1992. A family of human cdc2-related protein kinases. *EMBO J.* 11: 2909-2917.
3. Ershler, M., et al. 1993. Novel CDC2-related protein kinases produced in murine hematopoietic stem cells. *Gene* 124: 305-306.
4. Nagase, T., et al. 1998. Prediction of the coding sequences of unidentified human genes. XII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res.* 5: 355-364.
5. Marques, F., et al. 2000. A new subfamily of high molecular mass CDC2-related kinases with PITAI/VRE motifs. *Biochem. Biophys. Res. Commun.* 279: 832-837.

CHROMOSOMAL LOCATION

Genetic locus: Cdk12 (mouse) mapping to 11 D.

PRODUCT

CrkRS shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CrkRS siRNA (m): sc-44531 and CrkRS shRNA Plasmid (m): sc-44531-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

CrkRS shRNA (m) Lentiviral Particles is recommended for the inhibition of CrkRS expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CrkRS (N-16): sc-32643 is recommended as a control antibody for monitoring of CrkRS 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CrkRS gene expression knockdown using RT-PCR Primer: CrkRS (m)-PR: sc-44531-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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