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VRK3 siRNA (m): sc-155229

BACKGROUND

The vaccinia-related kinase (VRK) proteins consist of three Ser-Thr kinases, designated VRK1, VRK2 and VRK3. In the human kinome, VRK proteins function as upstream regulators of several transcription factors. VRK3 (vaccinia related kinase 3) is a 474 amino acid nuclear protein that contains one protein kinase domain and belongs to the serine/threonine protein kinase family. Widely expressed in human tissues, VRK3 is thought to regulate ERK (extracellular signal regulated kinases) activity by directly binding to MPKs (mitogen-activated protein kinase phosphatases), specifically vaccinia H1-related (VHR) phosphatase, thereby dephosphorylating and inactivating ERK in the nucleus. VRK3 exists as two alternatively spliced variants and is encoded by a gene located on human chromosome 19, which consists of around 63 million bases, over 1,400 genes and makes up over 2% of human genomic DNA.

REFERENCES

1. Nezu, J., et al. 1997. Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase. *Genomics* 45: 327-331.
2. Vega, F.M., et al. 2003. Expression of the VRK (vaccinia-related kinase) gene family of p53 regulators in murine hematopoietic development. *FEBS Lett.* 544: 176-180.
3. Nichols, R.J. and Traktman, P. 2004. Characterization of three paralogous members of the mammalian vaccinia related kinase family. *J. Biol. Chem.* 279: 7934-7946.
4. Blanco, S., et al. 2006. The subcellular localization of vaccinia-related kinase-2 (VRK2) isoforms determines their different effect on p53 stability in tumour cell lines. *FEBS J.* 273: 2487-2504.
5. Nichols, R.J., et al. 2006. The vaccinia-related kinases phosphorylate the N' terminus of BAF, regulating its interaction with DNA and its retention in the nucleus. *Mol. Biol. Cell* 17: 2451-2464.
6. Kang, T.H. and Kim, K.T. 2006. Negative regulation of ERK activity by VRK3-mediated activation of VHR phosphatase. *Nat. Cell Biol.* 8: 863-869.

CHROMOSOMAL LOCATION

Genetic locus: *Vrk3* (mouse) mapping to 7 B4.

PRODUCT

VRK3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VRK3 shRNA Plasmid (m): sc-155229-SH and VRK3 shRNA (m) Lentiviral Particles: sc-155229-V as alternate gene silencing products.

For independent verification of VRK3 (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-155229A, sc-155229B and sc-155229C.

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

APPLICATIONS

VRK3 siRNA (m) is recommended for the inhibition of VRK3 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 μ M in 66 μ 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

VRK3 (C-8): sc-398771 is recommended as a control antibody for monitoring of VRK3 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 goat anti-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor VRK3 gene expression knockdown using RT-PCR Primer: VRK3 (m)-PR: sc-155229-PR (20 μ l). 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 or our catalog for detailed protocols and support products.