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SANTA CRUZ BIOTECHNOLOGY, INC.

VRK1 siRNA (m): sc-155227

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Human vaccina-related kinases 1 and 2 (VRK1,2) are NLS-containing, serine/threonine poxvirus-related kinases that are similar to casein kinase-1 family members. These VRK kinases phosphorylate transcription factors related to stress responses, such as p53. As an upstream regulator of p53, VRK-1 is capable of phosphorylating phosvitin, casein, histone 2b and myelin basic protein. VRK1 co-localizes with ATF2 in the nucleus and can form a stable complex. VRK1 phosphorylates ATF2 mainly on Thr-73, stabilizing the ATF2 protein and increasing its intracellular level. VRK1 phosphorylates human p53 in Thr18 and disrupts p53-Mdm2 interaction *in vitro*. VRK1 phosphorylates c-Jun in Ser 63 and Ser 73 *in vitro* (the same residues targeted by the N-terminal kinase of c-Jun (JNK)), and activates c-Jun dependent transcription.

REFERENCES

1. Hunter, T. 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 80: 225-236.
2. 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.
3. Lopez-Borges, S., et al. 2000. The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2 binding site of the p53 tumour suppressor protein. Oncogene 19: 3656-3664.
4. Nichols, R.J., et al. 2004. Characterization of three paralogous members of the mammalian vaccinia related kinase family. J. Biol. Chem. 279: 7934-7946.
5. Boyle, K.A., et al. 2004. Members of a novel family of mammalian protein kinases complement the DNA-negative phenotype of a vaccinia virus ts mutant defective in the B1 kinase. J. Virol. 78: 1992-2005.
6. Sevilla, A., et al. 2004. Human vaccinia-related kinase 1 (VRK1) activates the ATF2 transcriptional activity by novel phosphorylation on Thr-73 and Ser-62 and cooperates with JNK. J. Biol. Chem. 279: 27458-27465.

CHROMOSOMAL LOCATION

Genetic locus: Vrk1 (mouse) mapping to 12 F1.

PRODUCT

VRK1 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 VRK1 shRNA Plasmid (m): sc-155227-SH and VRK1 shRNA (m) Lentiviral Particles: sc-155227-V as alternate gene silencing products.

For independent verification of VRK1 (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-155227A, sc-155227B and sc-155227C.

PROTOCOLS

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

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

VRK1 siRNA (m) is recommended for the inhibition of VRK1 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

VRK1 (A-11): sc-271061 is recommended as a control antibody for monitoring of VRK1 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 IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor VRK1 gene expression knockdown using RT-PCR Primer: VRK1 (m)-PR: sc-155227-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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