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JNK1 shRNA (h2) Lentiviral Particles: sc-44210-V

BACKGROUND

c-Jun N-terminal kinases (JNKs) phosphorylate and augment transcriptional activity of c-Jun. JNKs originate from three genes that yield 10 isoforms through alternative mRNA splicing, including JNK1a1, JNK1b1, JNK2a1, JNK2b1, and JNK3a1, which represent the p46 isoforms, and JNK1a2, JNK1b2, JNK2a2, JNK2b2, and JNK3b2, which represent the p54 isoforms. JNKs coordinate cell responses to stress and influence regulation of cell growth and transformation. The human JNK1 (PRKM8, SAPK1, MAPK8) gene maps to chromosome 10q11.22 and shares 83% amino acid identity with JNK2. JNK1 is necessary for normal activation and differentiation of CD4 helper T (TH) cells into TH1 and TH2 effector cells. Capsaicin activates JNK1 and p38 in ras-transformed human breast epithelial cells. Nitrogen oxides (NOx) upregulate JNK1 in addition to c-Fos, c-Jun, and other signaling kinases, including MEKK1 and p38.

REFERENCES

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2. Dong, C., et al. 1998. Defective T cell differentiation in the absence of Jnk1. *Science* 282: 2092-2095.
3. Potapova, O., et al. 2000. Inhibition of c-Jun N-terminal kinase 2 expression suppresses growth and induces apoptosis of human tumor cells in a p53-dependent manner. *Mol. Cell. Biol.* 20: 1713-1722.
4. Dong, C., et al. 2000. JNK is required for effector T-cell function but not for T-cell activation. *Nature* 405: 91-94.
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CHROMOSOMAL LOCATION

Genetic locus: MAPK8 (human) mapping to 10q11.22.

PRODUCT

JNK1 shRNA (h2) 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 JNK1 siRNA (h2): sc-44210 and JNK1 shRNA Plasmid (h2): sc-44210-SH as alternate gene silencing products.

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.

APPLICATIONS

JNK1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of JNK1 expression in human 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

JNK1 (G-4): sc-398989 is recommended as a control antibody for monitoring of 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 JNK1 gene expression knockdown using RT-PCR Primer: JNK1 (h2)-PR: sc-44210-PR (20 μ l, 437 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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.

PROTOCOLS

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