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c-Jun shRNA (h2) Lentiviral Particles: sc-44201-V

BACKGROUND

Genes belonging to the Jun and Fos oncogene families encode nuclear proteins that are found to be associated with a number of transcriptional complexes. The c-Jun protein is a major component of the transcription factor AP-1, originally shown to mediate phorbol ester tumor promoter (TPA)-induced expression of responsive genes through the TPA response element (TRE). The Jun proteins form homo- and heterodimers which bind the TRE, while Fos proteins are active only as heterodimers with any of the Jun proteins. Fos/Jun heterodimers have a much higher affinity for the TRE than Jun homodimers. Ha-Ras augments c-Jun activity and stimulates phosphorylation of its activation domain. An inhibitor of Fos/Jun function, termed IP-1, associates with Fos and Jun and is inactivated upon phosphorylation induced by the cAMP-dependent protein kinase A (PKA).

REFERENCES

1. Sambucetti, L.C., et al. 1986. The Fos protein complex is associated with DNA in isolated nuclei and binds to DNA cellulose. *Science* 234: 1417-1419.
2. Bohmann, D., et al. 1987. Human proto-oncogene c-Jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science* 238: 1386-1392.
3. Distel, R.J., et al. 1987. Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-Fos. *Cell* 49: 835-844.
4. Renz, M., et al. 1987. Chromatin association and DNA-binding properties of the c-Fos proto-oncogene product. *Nucleic Acids Res.* 15: 277-292.
5. Angel, P., et al. 1988. Oncogene Jun encodes a sequence-specific transactivator similar to AP-1. *Nature* 332: 166-171.

CHROMOSOMAL LOCATION

Genetic locus: JUN (human) mapping to 1p32.1.

PRODUCT

c-Jun shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 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 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see c-Jun siRNA (h2): sc-44201 and c-Jun shRNA Plasmid (h2): sc-44201-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.

PROTOCOLS

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

APPLICATIONS

c-Jun shRNA (h2) Lentiviral Particles is recommended for the inhibition of c-Jun 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 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

c-Jun (B-1): sc-166540 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 c-Jun gene expression knockdown using RT-PCR Primer: c-Jun (h2)-PR: sc-44201-PR (20 μ l, 464 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.