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SH2-B shRNA (h) Lentiviral Particles: sc-44095-V

BACKGROUND

SH2-B is a component of the signaling network involved in the regulation of cell shape and movement. SH2-B is related to the APS (adapter molecule containing PH and SH2 domains) family of adapter proteins, which characteristically contain a Pleckstrin homology (PH) domain, an SH2 domain and a tyrosine phosphorylation site. SH2-B is alternatively spliced to generate three distinct isoforms, SH2-B α , β and γ , that share an identical N-terminal sequence, including the PH domain, the SH2 domain, and multiple proline-rich motifs. The isoform SH2-B β contributes to the regulation of the Actin cytoskeleton as it associates with various tyrosine kinases in response to growth factor stimulation. Following PDGF stimulation, SH2-B β can directly interact with the PDGF receptor (PDGFR) where it is phosphorylated on tyrosine residues and functions as a signaling protein for the PDGFR pathway. In addition, SH2-B β is also a substrate for JAK2 and, thereby, mediates the cytoskeletal reorganization that is induced by the signaling pathways of various growth factors.

REFERENCES

1. Frank, S.J., Yi, W., Zhao, Y., Goldsmith, J.F., Gilliland, G., Jiang, J., Sakai, I. and Kraft, A.S. 1995. Regions of the JAK2 tyrosine kinase required for coupling to the growth hormone receptor. *J. Biol. Chem.* 270: 14776-14785.
2. Rui, L., Mathews, L.S., Hotta, K., Gustafson, T.A. and Carter-Su, C. 1997. Identification of SH2-B β as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol. Cell. Biol.* 17: 6633-6644.
3. Rui, L. and Carter-Su, C. 1998. Platelet-derived growth factor (PDGF) stimulates the association of SH2-B β with PDGF receptor and phosphorylation of SH2-B β . *J. Biol. Chem.* 273: 21239-21245.
4. Rui, L. and Carter-Su, C. 1999. Identification of SH2-B β as a potent cytoplasmic activator of the tyrosine kinase Janus kinase 2. *Proc. Natl. Acad. Sci. USA* 96: 7172-7177.

CHROMOSOMAL LOCATION

Genetic locus: SH2B1 (human) mapping to 16p11.2.

PRODUCT

SH2-B shRNA (h) 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 SH2-B siRNA (h): sc-44095 and SH2-B shRNA Plasmid (h): sc-44095-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

SH2-B shRNA (h) Lentiviral Particles is recommended for the inhibition of SH2-B 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

SH2-B (E-8): sc-393395 is recommended as a control antibody for monitoring of SH2-B 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 SH2-B gene expression knockdown using RT-PCR Primer: SH2-B (h)-PR: sc-44095-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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