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survivin shRNA (h2) Lentiviral Particles: sc-44197-V

BACKGROUND

The baculovirus protein p35 inhibits virally-induced apoptosis of invertebrate and mammalian cells and may function to impair the clearing of virally infected cells by the immune system of the host. This is accomplished at least in part by the ability of p35 to block both TNF- and FAS-mediated apoptosis through the inhibition of the ICE family of serine proteases. Two mammalian homologs of baculovirus p35, referred to as inhibitor of apoptosis protein (IAP) 1 and 2, share an amino-terminal baculovirus IAP repeat (BIR) motif and a carboxy-terminal RING finger. Although the c-IAPs do not directly associate with the TNF receptor (TNF-R), they efficiently block TNF-mediated apoptosis through their interaction with the downstream TNF-R effectors, TRAF1 and TRAF2. Additional IAP family members include ILP (for IAP-like protein) and survivin. ILP inhibits activated caspase-3, leading to the resistance of FAS-mediated apoptosis. Survivin (also designated TIAP) is expressed during the G₂/M phase of the cell cycle and associates with microtubules of the mitotic spindle. Increased caspase-3 activity is detected when a disruption of survivin-microtubule interactions occurs.

REFERENCES

- Hay, B.A., et al. 1994. Expression of baculovirus p35 prevents cell death in *Drosophila*. *Develop.* 120: 2121-2129.
- Beidler, D.R., et al. 1995. The baculovirus p35 protein inhibits Fas- and tumor necrosis factor-induced apoptosis. *J. Biol. Chem.* 270: 16526-16528.
- Bump, N.J., et al. 1995. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. *Science* 269: 1885-1888.
- Rothe, M., et al. 1995. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 83: 1243-1252.

CHROMOSOMAL LOCATION

Genetic locus: BIRC5 (human) mapping to 17q25.3.

PRODUCT

survivin 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 survivin siRNA (h2): sc-44197 and survivin shRNA Plasmid (h2): sc-44197-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

survivin shRNA (h2) Lentiviral Particles is recommended for the inhibition of survivin 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

survivin (C-6): sc-374616 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 survivin gene expression knockdown using RT-PCR Primer: survivin (h2)-PR: sc-44197-PR (20 μ l, 524 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.