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14-3-3 γ shRNA (h2) Lentiviral Particles: sc-44279-V

BACKGROUND

14-3-3 proteins regulate many cellular processes relevant to cancer biology, notably apoptosis, mitogenic signaling and cell-cycle checkpoints. Seven isoforms comprise this family of signaling intermediates, denoted 14-3-3 β , γ , ϵ , ζ , η , θ and σ . 14-3-3 proteins form dimers that present two binding sites for ligand proteins, thereby bringing together two proteins that may not otherwise associate. These ligands largely share a 14-3-3 consensus binding motif and exhibit serine/threonine phosphorylation. 14-3-3 proteins function in broad regulation of these ligand proteins, by cytoplasmic sequestration, occupation of interaction domains and import/export sequences, prevention of degradation, activation/repression of enzymatic activity and facilitation of protein modification, and thus loss of expression contributes to a vast array of pathogenic cellular activities.

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CHROMOSOMAL LOCATION

Genetic locus: YWHAG (human) mapping to 7q11.23.

PRODUCT

14-3-3 γ 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 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 14-3-3 γ siRNA (h2): sc-44279 and 14-3-3 γ shRNA Plasmid (h2): sc-44279-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

14-3-3 γ shRNA (h2) Lentiviral Particles is recommended for the inhibition of 14-3-3 γ 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

14-3-3 γ (C-16): sc-731 is recommended as a control antibody for monitoring of 14-3-3 γ 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 14-3-3 γ gene expression knockdown using RT-PCR Primer: 14-3-3 γ (h2)-PR: sc-44279-PR (20 μ l). 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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