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AHA-1 shRNA (h) Lentiviral Particles: sc-44863-V

BACKGROUND

AHA-1 stimulates the inherent ATPase activity of yeast and human HSP 90 and interacts with the cytoplasmic tail of vesicular stomatitis virus glycoprotein. AHA-1 regulates HSP 90 by influencing the conformational state of the "ATP lid" and consequent N-terminal dimerization. It is crucial for cell viability under non-optimal growth conditions when HSP 90 levels are limiting. AHA-1 is a cytosolic protein and may transiently interact with the endoplasmic reticulum. It can have an effect on one step in the endoplasmic to Golgi trafficking. AHA-1 is expressed in numerous tissues, including brain, heart, skeletal muscle and kidney and, at lower levels, in liver and placenta. It is induced by heat shock and treatment with the HSP 90 inhibitor 17-demethoxygeldanamycin.

REFERENCES

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- Hu, R.M., et al. 2000. Gene expression profiling in the human hypothalamus-pituitary-adrenal axis and full-length cDNA cloning. *Proc. Natl. Acad. Sci. USA* 97: 9543-9548.
- Sevier, C.S., et al. 2001. p38: A novel protein that associates with the vesicular stomatitis virus glycoprotein. *Biochem. Biophys. Res. Commun.* 287: 574-582.
- Panaretou, B., et al. 2002. Activation of the ATPase activity of HSP 90 by the stress-regulated cochaperone AHA-1. *Mol. Cell* 10: 1307-1318.
- Lotz, G.P., et al. 2003. AHA-1 binds to the middle domain of HSP 90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. *J. Biol. Chem.* 278: 17228-17235.

CHROMOSOMAL LOCATION

Genetic locus: AHSA1 (human) mapping to 14q24.3.

PRODUCT

AHA-1 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 AHA-1 siRNA (h): sc-44863 and AHA-1 shRNA Plasmid (h): sc-44863-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

AHA-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of AHA-1 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

AHA-1 (F-7): sc-166610 is recommended as a control antibody for monitoring of AHA-1 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 AHA-1 gene expression knockdown using RT-PCR Primer: AHA-1 (h)-PR: sc-44863-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.