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AHA-1 siRNA (h): sc-44864

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 affect 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-demeth-oxygeldanamycin.

REFERENCES

1. Zhang, Q.H., et al. 2000. Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34⁺ hematopoietic stem/progenitor cells. *Genome Res.* 10: 1546-1560.
2. 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.
3. 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.
4. 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.
5. 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 (mouse) mapping to 12 D2.

PRODUCT

AHA-1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AHA-1 shRNA Plasmid (m): sc-44864-SH and AHA-1 shRNA (m) Lentiviral Particles: sc-44864-V as alternate gene silencing products.

For independent verification of AHA-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44864A, sc-44864B and sc-44864C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AHA-1 siRNA (m) is recommended for the inhibition of AHA-1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AHA-1 gene expression knockdown using RT-PCR Primer: AHA-1 (m)-PR: sc-44864-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.