

AHSP siRNA (h): sc-60137

BACKGROUND

α -hemoglobin stabilizing protein (AHSP), also designated erythroid associated factor (ERAF), is an erythroid-specific protein that acts as a chaperone to prevent the aggregation of α -hemoglobin during normal erythroid cell development. It specifically protects free α -hemoglobin from precipitation in live cells and in solution. It forms a heterodimer with free α -hemoglobin, but not with β -hemoglobin or hemoglobin A ($\alpha 2\beta 2$). AHSP localizes to the cytoplasm and is expressed in the blood and bone marrow. The AHSP protein is down-regulated in transmissible spongiform encephalopathies (TSEs). AHSP may regulate pathological states of α -hemoglobin excess such as β -thalassemia, a group of hereditary disorders involving the decreased production of normal adult hemoglobin (HbA) that are characterized by a deficiency in the synthesis of β -globin chains.

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. Miele, G., et al. 2001. A novel erythroid-specific marker of transmissible spongiform encephalopathies. *Nat. Med.* 7: 361-364.
3. Kihm, A.J., et al. 2002. An abundant erythroid protein that stabilizes free α -haemoglobin. *Nature* 417: 758-763.
4. Gell, D., et al. 2002. Bio-physical characterization of the α -globin binding protein α -hemoglobin stabilizing protein. *J. Biol. Chem.* 277: 40602-40609.

CHROMOSOMAL LOCATION

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

PRODUCT

AHSP siRNA (h) 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 AHSP shRNA Plasmid (h): sc-60137-SH and AHSP shRNA (h) Lentiviral Particles: sc-60137-V as alternate gene silencing products.

For independent verification of AHSP (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60137A, sc-60137B and sc-60137C.

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

AHSP siRNA (h) is recommended for the inhibition of AHSP expression in human 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

AHSP (FL-102): sc-292377 is recommended as a control antibody for monitoring of AHSP 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 AHSP gene expression knockdown using RT-PCR Primer: AHSP (h)-PR: sc-60137-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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