SANTA CRUZ BIOTECHNOLOGY, INC.

Hemoglobin β siRNA (m): sc-35559

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The Power to Question

BACKGROUND

Hemoglobin (Hgb) is coupled to four iron-binding, methene-linked tetrapyrrole rings (heme). The α (16p13.3; 5'-ζ-pseudoζ-pseudoα2-pseudoα1-α2-α1-θ1-3') and β (11p15.4) globin loci determine the basic hemoglobin structure. The globin portion of Hgb consists of two α chains and two β chains arranged in pairs forming a tetramer. Each of the four globin chains covalently associates with a heme group. The bonds between α and β chains are weaker than between similar globin chains, thereby forming a cleavage plane that is important for oxygen binding and release. High affinity for oxygen occurs upon relaxation of the α1-β2 cleavage plane. When the two α1-β2 interfaces are closely bound, hemoglobin has a low affinity for oxygen. Hb A, which contains two α chains plus two β chains, comprises 97% of total circulating hemoglobin. The remaining 3% of total circulating hemoglobin is comprised of Hb A-2, which consists of two α chains plus two δ chains, and fetal hemoglobin (Hb F), which consists of two α chains together with two γ chains.

REFERENCES

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2. Goodbourn, S.E., et al. 1983. Molecular basis of length polymorphism in the human ζ-globin gene complex. Proc. Natl. Acad. Sci. USA 80: 5022-5026.
3. Giardina, B., et al. 1995. The multiple functions of hemoglobin. Crit. Rev. Biochem. Mol. Biol. 30: 165-196.
4. Adachi, K., et al. 2002. Assembly of human hemoglobin (Hb) β- and γ-globin chains expressed in a cell-free system with α-globin chains to form Hb A and Hb F. J. Biol. Chem. 277: 13415-13420.
5. Feng, L., et al. 2004. Molecular mechanism of AHSP-mediated stabilization of α-hemoglobin. Cell 119: 629-640.
6. Sudha, R., et al. 2004. Linkage of interactions in sickle hemoglobin fiber assembly: inhibitory effect emanating from mutations in the AB region of the α-chain is annulled by a mutation at its EF corner. J. Biol. Chem. 279: 20018-20027.

CHROMOSOMAL LOCATION

Genetic locus: Hbb-b1 (mouse) mapping to 7 E3.

PRODUCT

Hemoglobin β 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hemoglobin β shRNA Plasmid (m): sc-35559-SH and Hemoglobin β shRNA (m) Lentiviral Particles: sc-35559-V as alternate gene silencing products.

For independent verification of Hemoglobin β (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-35559A, sc-35559B and sc-35559C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Hemoglobin β siRNA (m) is recommended for the inhibition of Hemoglobin β 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 µM in 66 µl. 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

Hemoglobin β/γ/δ/ε (A-8): sc-390668 is recommended as a control antibody for monitoring of Hemoglobin β 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Hemoglobin β gene expression knockdown using RT-PCR Primer: Hemoglobin β (m)-PR: sc-35559-PR (20 µl). 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 for detailed protocols and support products.