

# Hemoglobin $\alpha$ siRNA (h): sc-41230

## BACKGROUND

Hemoglobin (Hgb) is coupled to four iron-binding, methene-linked tetrapyrrole rings (heme). The  $\alpha$  (16p13.3; 5'- $\zeta$ -pseudo $\zeta$ -pseudo $\alpha$ 2-pseudo $\alpha$ 1- $\alpha$ 2- $\alpha$ 1- $\theta$ 1-3') and  $\beta$  (11p15.5) globin loci determine the basic Hgb structure. The globin portion of Hgb consists of two  $\alpha$  chains and two  $\beta$  chains arranged in pairs forming a tetramer. Each of the four globin chains covalently associates with a heme group. The bonds between  $\alpha$  and  $\beta$  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  $\alpha$ 1- $\beta$ 2 cleavage plane. When the two  $\alpha$ 1- $\beta$ 2 interfaces are closely bound, Hgb has a low affinity for oxygen. Hb A, which contains two  $\alpha$  chains plus two  $\beta$  chains, comprises 97% of total circulating hemoglobin. The remaining 3% of total circulating hemoglobin is comprised of Hb A-2, which consists of two  $\alpha$  chains plus two  $\delta$  chains, and fetal hemoglobin (Hb F), which consists of two  $\alpha$  chains together with two  $\gamma$  chains.

## REFERENCES

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2. Goodbourn, S.E., et al. 1983. Molecular basis of length polymorphism in the human  $\zeta$ -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)  $\beta$ - and  $\gamma$ -globin chains expressed in a cell-free system with  $\alpha$ -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  $\alpha$ -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  $\alpha$ -chain is annulled by a mutation at its EF corner. *J. Biol. Chem.* 279: 20018-20027.
7. Baudin-Creuz, V., et al. 2004. Transfer of human  $\alpha$ - to  $\beta$ -hemoglobin via its chaperone protein: evidence for a new state. *J. Biol. Chem.* 279: 36530-36533.

## CHROMOSOMAL LOCATION

Genetic locus: HBA1 (human) mapping to 16p13.3.

## PRODUCT

Hemoglobin  $\alpha$  siRNA (h) is a target-specific 19-25 nt siRNA designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hemoglobin  $\alpha$  shRNA Plasmid (h): sc-41230-SH and Hemoglobin  $\alpha$  shRNA (h) Lentiviral Particles: sc-41230-V as alternate gene silencing products.

## RESEARCH USE

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

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

## APPLICATIONS

Hemoglobin  $\alpha$  siRNA (h) is recommended for the inhibition of Hemoglobin  $\alpha$  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  $\mu$ M in 66  $\mu$ 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

Hemoglobin  $\alpha$  (D-4): sc-514378 is recommended as a control antibody for monitoring of Hemoglobin  $\alpha$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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  $\alpha$  gene expression knockdown using RT-PCR Primer: Hemoglobin  $\alpha$  (h)-PR: sc-41230-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.