SANTA CRUZ BIOTECHNOLOGY, INC.

# Hephaestin siRNA (h): sc-60780

## BACKGROUND

Hephaestin is a single-pass type I membrane protein that belongs to the multicopper oxidase family of proteins. Hephaestin, a copper-dependant ferroxidase protein, is crucial for iron exiting intestinal enterocytes into the circulation. It mediates the movement of iron across the basolateral membrane in conjunction with ferroportin 1. This is an important link between iron and copper metabolism in mammalian systems, as copper deficiency leads to reduced hephaestin and reduced iron absorption resulting in anemia. Hephaestin can bind six copper ions per monomer and is regulated by the homeobox transcription factor CDX2. Increased levels of iron leads to an increase in CDX2 expression and thus Hephaestin. Hephaestin is primarily detected in the intestine, but is also expressed in colon, breast, bone trabecural cells and fibroblasts.

## REFERENCES


1. Anderson, G.J., et al. 2005. Recent advances in intestinal iron transport. Curr. Gastroenterol. Rep. 7: 365-372.
2. Anderson, G.J., et al. 2005. Mechanisms of haem and non-haem iron absorption: lessons from inherited disorders of iron metabolism. Biometals 18: 339-348.
3. Petrak, J., et al. 2005. Hephaestin—a ferroxidase of cellular iron export. Int. J. Biochem. Cell Biol. 37: 1173-1178.
4. Gleeson, F., et al. 2005. Duodenal Dcytb and Hephaestin mRNA expression are not significantly modulated by variations in body iron homeostasis. Blood Cells Mol. Dis. 35: 303-308.
5. Reeves, P.G., et al. 2005. Repletion of copper-deficient rats with dietary copper restores duodenal Hephaestin protein and iron absorption. Exp. Biol. Med. 230: 320-325.
6. Hinoi, T., et al. 2005. CDX2-regulated expression of iron transport protein Hephaestin in intestinal and colonic epithelium. Gastroenterology 128: 946-961.
7. Reeves, P.G., et al. 2005. Dietary copper deficiency reduces iron absorption and duodenal enterocyte Hephaestin protein in male and female rats. J. Nutr. 135: 92-98.


## CHROMOSOMAL LOCATION

Genetic locus: HEPH (human) mapping to Xq12.

## PRODUCT

Hephaestin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hephaestin shRNA Plasmid (h): sc-60780-SH and Hephaestin shRNA (h) Lentiviral Particles: sc-60780-V as alternate gene silencing products.

For independent verification of Hephaestin (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-60780A, sc-60780B and sc-60780C.

## 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

Hephaestin siRNA (h) is recommended for the inhibition of Hephaestin 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 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

Hephaestin (C-7): sc-365365 is recommended as a control antibody for monitoring of Hephaestin 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 Hephaestin gene expression knockdown using RT-PCR Primer: Hephaestin (h)-PR: sc-60780-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.