

HCP1 siRNA (h): sc-72314

BACKGROUND

HCP1 (heme carrier protein 1), also known as proton-coupled folate transporter (PCFT), is a multi-pass transmembrane protein that is expressed in the small intestine. It is predominantly found in the duodenum and the jejunum localizing to the apical brush border. HCP1 is required for normal folate absorption in the intestine and is associated with folate homeostasis. HCP1 mediates the transport of folate and functions most optimally at a low extracellular pH of approximately 5.5. HCP1 functions independently of Na⁺ and is insensitive to membrane potential. It exhibits high affinity for folic acid and low affinity for the PT523 antifolate. HCP1 is posttranslationally regulated by iron levels in the duodenum. During iron deficiency, HCP1 localizes to the apical membrane; however, iron excess causes HCP1 to localize in the cytoplasm. Sulfasalazine is a potent inhibitor of HCP1. Mutations in the gene encoding HCP1 can result in the autosomal recessive disorder hereditary folate malabsorption (HFM).

REFERENCES

1. Rouault, T.A. 2005. The intestinal heme transporter revealed. *Cell* 122: 649-651.
2. Shayeghi, M., et al. 2005. Identification of an intestinal heme transporter. *Cell* 122: 789-801.
3. Qiu, A., et al. 2006. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* 127: 917-928.
4. Latunde-Dada, G.O., et al. 2006. Heme carrier protein 1 (HCP1): Expression and functional studies in cultured cells. *FEBS Lett.* 580: 6865-6870.
5. Latunde-Dada, G.O., et al. 2006. Recent advances in mammalian heme transport. *Trends Biochem. Sci.* 31: 182-188.
6. Sharma, S., et al. 2007. Heme carrier protein 1 (HCP1) expression and functional analysis in the retina and retinal pigment epithelium. *Exp. Cell Res.* 313: 1251-1259.
7. Qiu, A., et al. 2007. Rodent intestinal folate transporters (SLC46A1): secondary structure, functional properties, response to dietary folate restriction. *Am. J. Physiol., Cell Physiol.* 293: C1669-C1678.

CHROMOSOMAL LOCATION

Genetic locus: SLC46A1 (human) mapping to 17q11.2.

PRODUCT

HCP1 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 HCP1 shRNA Plasmid (h): sc-72314-SH and HCP1 shRNA (h) Lentiviral Particles: sc-72314-V as alternate gene silencing products.

For independent verification of HCP1 (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-72314A, sc-72314B and sc-72314C.

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

HCP1 siRNA (h) is recommended for the inhibition of HCP1 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

HCP1 (B-4): sc-393460 is recommended as a control antibody for monitoring of HCP1 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HCP1 gene expression knockdown using RT-PCR Primer: HCP1 (h)-PR: sc-72314-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.