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cytoglobin siRNA (h): sc-45547

BACKGROUND

Hemoglobin, myoglobin, neuroglobin, and cytoglobin belong to the globin family, porphyrin-containing proteins that function in oxygen transport and storage. Myoglobin contributes to intracellular oxygen storage and transcellular-facilitated diffusion of oxygen in skeletal and cardiac muscle. Neuroglobin is an oxidative stress-responsive sensor for signal transduction in the brain. Hemoglobin contributes to oxygen storage and diffusion of oxygen in blood tissue. Cytoglobin (also designated histogloblin or STAP), is a ubiquitous protein that facilitates diffusion of oxygen through tissues and acts as a scavenger for nitric oxide or other reactive oxygen species. It binds O₂ via its heme and also has a protective function during oxidative stress. Cytoglobin, a hexacoordinate hemoglobin, shares less than 30% identity with other human hemoglobins and is widely expressed in a wide array of tissues including fibroblasts and nerve cell populations.

REFERENCES

1. Trent, J.T., 3rd and Hargrove M.S. 2002. A ubiquitously expressed human hexacoordinate hemoglobin. *J. Biol. Chem.* 277: 19538-19545.
2. de Sanctis, D., et al. 2004. Mapping protein matrix cavities in human cytoglobin through Xe atom binding. *Biochem. Biophys. Res. Commun.* 316: 1217-1221.
3. Fago, A., et al. 2004. Allosteric regulation and temperature dependence of oxygen binding in human neuroglobin and cytoglobin. *Molecular mechanisms and physiological significance. J. Biol. Chem.* 279: 44417-44426.
4. Weiland, T.R., et al. 2004. Bis-histidyl hexacoordination in hemoglobins facilitates heme reduction kinetics. *J. Am. Chem. Soc.* 126: 11930-11935.
5. Fordel, E., et al. 2004. Cytoglobin expression is upregulated in all tissues upon hypoxia: an *in vitro* and *in vivo* study by quantitative real-time PCR. *Biochem. Biophys. Res. Commun.* 319: 342-348.

CHROMOSOMAL LOCATION

Genetic locus: CYGB (human) mapping to 17q25.1.

PRODUCT

cytoglobin 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 cytoglobin shRNA Plasmid (h): sc-45547-SH and cytoglobin shRNA (h) Lentiviral Particles: sc-45547-V as alternate gene silencing products.

For independent verification of cytoglobin (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-45547A, sc-45547B and sc-45547C.

PROTOCOLS

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

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

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

cytoglobin (D-7): sc-365246 is recommended as a control antibody for monitoring of cytoglobin 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 cytoglobin gene expression knockdown using RT-PCR Primer: cytoglobin (h)-PR: sc-45547-PR (20 μ l, 564 bp). 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.