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cytoglobin shRNA (m) Lentiviral Particles: sc-45548-V

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), 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

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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. 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.
4. Kugelstadt, D., et al. 2004. Neuroglobin, cytoglobin, and a novel, eye-specific globin from chicken. *Biochem. Biophys. Res. Commun.* 325: 719-725.
5. 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.
6. Weiland, T.R., et al. 2004. Bis-histidyl hexacoordination in hemoglobins facilitates heme reduction kinetics. *J. Am. Chem. Soc.* 126: 11930-11935.

CHROMOSOMAL LOCATION

Genetic locus: *Cygb* (mouse) mapping to 11 E2.

PRODUCT

cytoglobin shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see cytoglobin siRNA (m): sc-45548 and cytoglobin shRNA Plasmid (m): sc-45548-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

cytoglobin shRNA (m) Lentiviral Particles is recommended for the inhibition of cytoglobin expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

cytoglobin (H-16): sc-34598 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor cytoglobin gene expression knockdown using RT-PCR Primer: cytoglobin (m)-PR: sc-45548-PR (20 µl, 491 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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