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Heme Oxygenase 1 shRNA (m2) Lentiviral Particles: sc-44307-V

BACKGROUND

Heme oxygenases are microsomal enzymes that cleave heme to produce the antioxidant biliverdin, inorganic iron and carbon monoxide (CO). The activity of Heme Oxygenase 1 (HO-1), also designated HSP 32, is highly inducible in response to numerous stimuli, including heme, heavy metals, hormones and oxidative stress. Heme Oxygenase 2, in contrast, appears to be constitutively expressed in mammalian tissues. Heme Oxygenase 2 is involved in the production of carbon monoxide (CO) in brain, where CO is thought to act as a neurotransmitter. The CO signaling system closely parallels the signaling pathway involving nitric oxide, and regulation of the two systems is closely linked. Heme Oxygenase 3 is found in the spleen, liver, thymus, prostate, heart, kidney, brain and testis. A poor heme catalyst, Heme Oxygenase 3 has two heme regulatory motifs that may be involved in heme binding.

REFERENCES

1. Maines, M.D. 1988. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J.* 2: 2557-2568.
2. Rodgers, P.A., et al. 1990. Developmental biology of heme oxygenase. *Clin. Perinatol.* 17: 275-291.
3. Alam, J., et al. 1994. Isolation and characterization of the mouse heme oxygenase-1 gene. Distal 5' sequences are required for induction by heme or heavy metals. *J. Biol. Chem.* 269: 1001-1009.
4. Maines, M.D. 1997. The heme oxygenase system; a regulator of second messenger gases. *Ann. Rev. Pharmacol. Toxicol.* 37: 517-554.
5. McCoubrey, W.K., et al. 1997. Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. *Eur. J. Biochem.* 247: 725-732.

CHROMOSOMAL LOCATION

Genetic locus: Hmx1 (mouse) mapping to 8 C1.

PRODUCT

Heme Oxygenase 1 shRNA (m2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 5 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 Heme Oxygenase 1 siRNA (m2): sc-44307 and Heme Oxygenase 1 shRNA Plasmid (m2): sc-44307-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.

PROTOCOLS

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

APPLICATIONS

Heme Oxygenase 1 shRNA (m2) Lentiviral Particles is recommended for the inhibition of Heme Oxygenase 1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

Heme Oxygenase 1 (F-4): sc-390991 is recommended as a control antibody for monitoring of Heme Oxygenase 1 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 Heme Oxygenase 1 gene expression knockdown using RT-PCR Primer: Heme Oxygenase 1 (m2)-PR: sc-44307-PR (20 μ l, 522 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.