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GRP 78 shRNA (m2) Lentiviral Particles: sc-44303-V

BACKGROUND

The HSP 70 family is composed of four highly conserved proteins: HSP 70, HSC 70, GRP 75 and GRP 78. These proteins serve a variety of roles: they act as molecular chaperones facilitating the assembly of multi-protein complexes, participate in the translocation of polypeptides across cell membranes and to the nucleus, and aid in the proper folding of nascent polypeptide chains. All members of the family, except HSP 70, are constitutively expressed in primate cells. HSP 70 expression is strongly induced in response to heat stress. HSP 70 and HSC 70 play key roles in the cytosolic endoplasmic reticulum and mitochondrial import machinery and are found in both the cytosol and nucleus of mammalian cells. Both HSP 70 and HSC 70 are involved in the chaperoning of nascent polypeptide chains and in protecting cells against the accumulation of improperly folded proteins. GRP 78 is localized in the endoplasmic reticulum, where it receives imported secretory proteins and is involved in the folding and translocation of nascent peptide chains. GRP 75 expression is restricted to the mitochondrial matrix and aids in the translocation and folding of nascent polypeptide chains of both nuclear and mitochondrial origin. GRP 75 and GRP 78 are unresponsive to heat stress and are induced by glucose deprivation. It has been postulated that members of the HSP 70 family act as force-generating motors, relying on the hydrolysis of ATP for their activity.

REFERENCES

1. Martin, J., et al. 1992. Prevention of protein denaturation under heat stress by the chaperonin HSP 60. *Science* 258: 995-998.
2. Hatayama, T., et al. 1992. Effects of low culture temperature on the induction of HSP 70 mRNA and the accumulation of HSP 70 and HSP 105 in mouse FM3A cells. *J. Biochem.* 111: 484-490.
3. Bhattacharyya, T., et al. 1995. Cloning and subcellular localization of human mitochondrial HSP 70. *J. Biol. Chem.* 270: 1705-1710.
4. Haas, I.G. 1995. Protein-mediated protein maturation in eukaryotes. *FEBS Lett.* 369: 72-75.

CHROMOSOMAL LOCATION

Genetic locus: Hspa5 (mouse) mapping to 2 B.

PRODUCT

GRP 78 shRNA (m2) 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 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 GRP 78 siRNA (m2): sc-44303 and GRP 78 shRNA Plasmid (m2): sc-44303-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

GRP 78 shRNA (m2) Lentiviral Particles is recommended for the inhibition of GRP 78 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

GRP 78 (A-10): sc-376768 is recommended as a control antibody for monitoring of GRP 78 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GRP 78 gene expression knockdown using RT-PCR Primer: GRP 78 (m2)-PR: sc-44303-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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

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