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ORP150 siRNA (m): sc-151324

BACKGROUND

ORP150, also known as HYOU1 (hypoxia upregulated 1), is a 999 amino acid protein that localizes to the lumen of the endoplasmic reticulum (ER) and is a member of the heat shock protein 70 (HSP 70) family. ORP150 is highly expressed in tissues that have well-developed ERs and a large number of secretory proteins (such as liver and pancreas). It is expressed at lower levels in kidney and brain, and plays an essential role in cytoprotective cellular responses to hypoxia (oxygen deprivation). Specifically, ORP150 participates in protein folding and secretion in the ER and functions to protect cells from hypoxia-induced apoptosis, thereby playing a crucial role in cell survival. ORP150 expression is upregulated in a variety of tumors, such as breast cancer, suggesting an important role in tumorigenesis. The gene encoding ORP150 has two translation initiation sites, resulting in a truncated transcript that lacks an ER signal peptide, but is thought to function as a housekeeping protein in the cytoplasm.

REFERENCES

- Ikeda, J., et al. 1997. Cloning and expression of cDNA encoding the human 150 kDa oxygen-regulated protein, ORP150. *Biochem. Biophys. Res. Commun.* 230: 94-99.
- Ozawa, K., et al. 1999. 150-kDa oxygen-regulated protein (ORP150) suppresses hypoxia-induced apoptotic cell death. *J. Biol. Chem.* 274: 6397-6404.
- Bando, Y., et al. 2000. 150-kDa oxygen-regulated protein (ORP150) functions as a novel molecular chaperone in MDCK cells. *Am. J. Physiol., Cell Physiol.* 278: C1172-C1182.

CHROMOSOMAL LOCATION

Genetic locus: Hyou1 (mouse) mapping to 9 A5.2.

PRODUCT

ORP150 siRNA (m) 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 ORP150 shRNA Plasmid (m): sc-151324-SH and ORP150 shRNA (m) Lentiviral Particles: sc-151324-V as alternate gene silencing products.

For independent verification of ORP150 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-151324A, sc-151324B and sc-151324C.

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

ORP150 siRNA (m) is recommended for the inhibition of ORP150 expression in mouse 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

ORP150 (A-3): sc-398224 is recommended as a control antibody for monitoring of ORP150 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 ORP150 gene expression knockdown using RT-PCR Primer: ORP150 (m)-PR: sc-151324-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Jung, T.W., et al. 2012. SIRT1 attenuates palmitate-induced endoplasmic reticulum stress and Insulin resistance in HepG2 cells via induction of oxygen-regulated protein 150. *Biochem. Biophys. Res. Commun.* 422: 229-232.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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