

REDD-1 siRNA (m): sc-45807

BACKGROUND

REDD-1, also designated DNA-damage-inducible transcript 4, dig2 or RTP801, is thought to function in the regulation of reactive oxygen species (ROS). REDD-1 expression has also been linked to apoptosis, Ab toxicity and the pathogenesis of ischemic diseases. As an HIF-1-responsive gene, REDD-1 exhibits strong hypoxia-dependent upregulation in ischemic cells of neuronal origin. In response to stress due to DNA damage and glucocorticoid treatment, REDD-1 is upregulated at the transcriptional level. REDD-1 negatively regulates the mammalian target of Rapamycin (mTOR), a serine/threonine kinase often referred to as FRAP. It is crucial in the coupling of extra- and intracellular cues to FRAP regulation. The absence of REDD-1 is associated with the development of retinopathy, a major cause of blindness.

REFERENCES

- Shoshani, T., et al. 2002. Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. *Mol. Cell. Biol.* 22: 2283-2293.
- Kim, J.R., et al. 2003. Identification of Amyloid β -peptide responsive genes by cDNA microarray technology: involvement of RTP801 in Amyloid β -peptide toxicity. *Exp. Mol. Med.* 35: 403-411.
- Brugarolas, J., et al. 2004. Regulation of mTOR function in response to hypoxia by REDD-1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev.* 18: 2893-2904.
- Lee, M., et al. 2004. Sp1-dependent regulation of the RTP801 promoter and its application to hypoxia-inducible VEGF plasmid for ischemic disease. *Pharm. Res.* 21: 736-741.
- Corradetti, M.N., et al. 2005. The stress-induced proteins RTP801 and RTP801L are negative regulators of the mammalian target of Rapamycin pathway. *J. Biol. Chem.* 280: 9769-9772.
- Schwarzer, R., et al. 2005. REDD-1 integrates hypoxia-mediated survival signaling downstream of phosphatidylinositol 3-kinase. *Oncogene* 24: 1138-1149.

CHROMOSOMAL LOCATION

Genetic locus: Ddit4 (mouse) mapping to 10 B4.

PRODUCT

REDD-1 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 REDD-1 shRNA Plasmid (m): sc-45807-SH and REDD-1 shRNA (m) Lentiviral Particles: sc-45807-V as alternate gene silencing products.

For independent verification of REDD-1 (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-45807A, sc-45807B and sc-45807C.

RESEARCH USE

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

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

REDD-1 siRNA (m) is recommended for the inhibition of REDD-1 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

REDD-1 (B-3): sc-376671 is recommended as a control antibody for monitoring of REDD-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 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 REDD-1 gene expression knockdown using RT-PCR Primer: REDD-1 (m)-PR: sc-45807-PR (20 μ l, 411 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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