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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

REDD-1 shRNA (m) Lentiviral Particles: sc-45807-V

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

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- 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.
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- 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 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 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 REDD-1 siRNA (m): sc-45807 and REDD-1 shRNA Plasmid (m): sc-45807-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

REDD-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of REDD-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

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 (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 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 $55-60^\circ$ 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.

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