SANTA CRUZ BIOTECHNOLOGY, INC.

REDD-1 siRNA (h): sc-45806

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.

CHROMOSOMAL LOCATION

Genetic locus: DDIT4 (human) mapping to 10q22.1.

PRODUCT

REDD-1 siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see REDD-1 shRNA Plasmid (h): sc-45806-SH and REDD-1 shRNA (h) Lentiviral Particles: sc-45806-V as alternate gene silencing products.

For independent verification of REDD-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45806A, sc-45806B and sc-45806C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

REDD-1 siRNA (h) is recommended for the inhibition of REDD-1 expression in human 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 µM in 66 µl. 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 (A-4): sc-271158 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 (h)-PR: sc-45806-PR (20 µl, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Jin, H.O., et al. 2013. Sustained overexpression of REDD-1 leads to Akt activation involved in cell survival. Cancer Lett. 336: 319-324.
2. Wang, S., et al. 2015. ATF4 gene network mediates cellular response to the anticancer PAD inhibitor YW3-56 in triple-negative breast cancer cells. Mol. Cancer Ther. 14: 877-888.
3. Yun, S.M., et al. 2016. Melatonin enhances arsenic trioxide-induced cell death via sustained upregulation of REDD-1 expression in breast cancer cells. Mol. Cell. Endocrinol. 422: 64-73.
4. Jin, H.O., et al. 2021. Amino acid deprivation induces AKT activation by inducing GCN2/ATF4/REDD1 axis. Cell Death Dis. 12: 1127.
5. Jang, S.K., et al. 2021. Inhibition of mTORC1 through ATF4-induced REDD1 and Sestrin2 expression by Metformin. BMC Cancer 21: 803.
6. Park, M., et al. 2021. REDD1 is a determinant of low-dose metronomic doxorubicin-elicited endothelial cell dysfunction through downregulation of VEGFR-2/3 expression. Exp. Mol. Med. 53: 1612-1622.
7. Tang, Y., et al. 2022. Advanced single-cell pooled CRISPR screening identifies C19orf53 required for cell proliferation based on mTORC1 regulators. Cell Biol. Toxicol. 38: 43-68.

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.