

USP48 siRNA (h): sc-76865

BACKGROUND

The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. Through the use of a wide range of enzymes that can add or remove ubiquitin, the Ub pathway controls many intracellular processes such as signal transduction, transcriptional activation and cell cycle progression. USP48 (ubiquitin specific peptidase 48), also known as USP31 or RAP1GA1 is a widely expressed 1,035 amino acid protein that belongs to the peptidase C19 family of proteins. Containing three DUSP domains and one ubiquitin-like domain, USP48 functions as a deubiquitinating enzyme that cleaves ubiquitin residues from both ubiquitinated proteins and ubiquitin-fused precursors, thereby saving these proteins from proteasomal degradation. By reversing the ubiquitination of proteins such as TRAF2 and TRAF6, USP48 plays an important role in the regulation of NF κ B activation. Seven isoforms of USP48 are expressed due to alternative splicing events.

REFERENCES

1. Wilkinson, K.D. 1997. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J.* 11: 1245-1256.
2. Puente, X.S., et al. 2003. Human and mouse proteases: a comparative genomic approach. *Nat. Rev. Genet.* 4: 544-558.
3. Lockhart, P.J., et al. 2004. Identification of the human ubiquitin specific protease 31 (USP31) gene: structure, sequence and expression analysis. *DNA Seq.* 15: 9-14.
4. Quesada, V., et al. 2004. Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. *Biochem. Biophys. Res. Commun.* 314: 54-62.
5. Hatano, Y., et al. 2004. PARK6-linked autosomal recessive early-onset parkinsonism in Asian populations. *Neurology* 63: 1482-1485.
6. Wullaert, A., et al. 2006. Ubiquitin: tool and target for intracellular NF κ B inhibitors. *Trends Immunol.* 27: 533-540.
7. Tzimas, C., et al. 2006. Human ubiquitin specific protease 31 is a deubiquitinating enzyme implicated in activation of nuclear factor- κ B. *Cell. Signal.* 18: 83-92.

CHROMOSOMAL LOCATION

Genetic locus: USP48 (human) mapping to 1p36.12.

PRODUCT

USP48 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see USP48 shRNA Plasmid (h): sc-76865-SH and USP48 shRNA (h) Lentiviral Particles: sc-76865-V as alternate gene silencing products.

For independent verification of USP48 (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-76865A, sc-76865B and sc-76865C.

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

USP48 siRNA (h) is recommended for the inhibition of USP48 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 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

USP48 (5C2): sc-100635 is recommended as a control antibody for monitoring of USP48 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 USP48 gene expression knockdown using RT-PCR Primer: USP48 (h)-PR: sc-76865-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

1. Cetkovská, K., et al. 2017. Ubiquitin-specific peptidase 48 regulates Mdm2 protein levels independent of its deubiquitinase activity. *Sci. Rep.* 7: 43180.

RESEARCH USE

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