

USP14 siRNA (m): sc-76818

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. USP14 (ubiquitin specific peptidase 14), also known as TGT (tRNA-guanine transglycosylase), is a cytoplasmic protein that belongs to the ubiquitin-specific processing family of deubiquitinating enzymes. Existing as a homodimer within the cell, USP14 functions to cleave ubiquitin residues from both ubiquitinated proteins and ubiquitin-fused precursors, thereby saving these proteins from proteasomal degradation. In mice, defects or mutations in the gene encoding USP14 cause retarded growth or fetal death, indicating that USP14 plays a key role in early developmental processes. Multiple isoforms of USP14 are expressed due to alternative splicing events.

REFERENCES

1. Deshpande, K.L., et al. 1996. Cloning and characterization of cDNA encoding the rabbit tRNA-guanine transglycosylase 60-kilodalton subunit. *Arch. Biochem. Biophys.* 326: 1-7.
2. D'Andrea, A. and Pellman, D. 1998. Deubiquitinating enzymes: a new class of biological regulators. *Crit. Rev. Biochem. Mol. Biol.* 33: 337-352.
3. Borodovsky, A., et al. 2001. A novel active site-directed probe specific for deubiquitylating enzymes reveals proteasome association of USP14. *EMBO J.* 20: 5187-5196.

CHROMOSOMAL LOCATION

Genetic locus: *Usp14* (mouse) mapping to 18 A1.

PRODUCT

USP14 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 USP14 shRNA Plasmid (m): sc-76818-SH and USP14 shRNA (m) Lentiviral Particles: sc-76818-V as alternate gene silencing products.

For independent verification of USP14 (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-76818A, sc-76818B and sc-76818C.

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

USP14 siRNA (m) is recommended for the inhibition of USP14 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

USP14 (6E6): sc-100630 is recommended as a control antibody for monitoring of USP14 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 USP14 gene expression knockdown using RT-PCR Primer: USP14 (m)-PR: sc-76818-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. Liu, N., et al. 2016. Ubiquitin-specific protease 14 regulates cardiac hypertrophy progression by increasing GSK-3 β phosphorylation. *Biochem. Biophys. Res. Commun.* 478: 1236-1241.

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.