

USP25 siRNA (m): sc-76830

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. USP25 (ubiquitin specific peptidase 25), also known as USP21, is a 1,087 amino acid member of the peptidase C19 family. Expressed at high amounts in pancreas, kidney, skeletal muscle, testis, liver, heart, brain and lung, USP25 associates with the 26S Proteasome and functions as a deubiquitinating enzyme in the Ub proteolytic pathway. Due to alternative splicing events, USP25 is expressed as two short, ubiquitously expressed isoforms and one long, muscle-specific isoform. The long isoform of USP25 is upregulated in myogenesis and is implicated in the pathogenesis of severe myopathies.

REFERENCES

1. Valero, R., et al. 1999. USP25, a novel gene encoding a deubiquitinating enzyme, is located in the gene-poor region 21q11.2. *Genomics* 62: 395-405.
2. Groet, J., et al. 2000. Narrowing of the region of allelic loss in 21q11-21 in squamous non-small cell lung carcinoma and cloning of a novel ubiquitin-specific protease gene from the deleted segment. *Genes Chromosomes Cancer* 27: 153-161.
3. Valero, R., et al. 2001. Characterization of alternatively spliced products and tissue-specific isoforms of USP28 and USP25. *Genome Biol.* 2: RESEARCH0043.

CHROMOSOMAL LOCATION

Genetic locus: *Usp25* (mouse) mapping to 16 C3.1.

PRODUCT

USP25 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 USP25 shRNA Plasmid (m): sc-76830-SH and USP25 shRNA (m) Lentiviral Particles: sc-76830-V as alternate gene silencing products.

For independent verification of USP25 (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-76830A, sc-76830B and sc-76830C.

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

USP25 siRNA (m) is recommended for the inhibition of USP25 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

USP25 (C-5): sc-398414 is recommended as a control antibody for monitoring of USP25 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 USP25 gene expression knockdown using RT-PCR Primer: USP25 (m)-PR: sc-76830-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. Ding, C., et al. 2017. Chloroquine attenuates lipopolysaccharide-induced inflammatory responses through upregulation of USP25. *Can. J. Physiol. Pharmacol.* 95: 481-491.

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.