

USP15 siRNA (m): sc-76820

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. USP15 (ubiquitin specific peptidase 15), also known as UNPH4, is a member of the peptidase C19 family of proteins. Expressed in kidney, liver, placenta, ovary, lung, thymus, heart and skeletal muscle, USP15 localizes to the cytoplasm and the nucleus, contains one DUSP domain and functions as a deubiquitinating enzyme that cleaves ubiquitin residues from both ubiquitinated proteins and ubiquitin-fused precursors, thereby saving these proteins from proteasomal degradation. Via its DUSP domain, USP15 plays a role in the regulation of the COP9 signalosome (CSN) complex. Three isoforms exist for USP15 due to alternative splicing events.

REFERENCES

1. Nagase, T., et al. 1998. Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. DNA Res. 5: 31-39.
2. Baker, R.T., et al. 1999. Identification, functional characterization, and chromosomal localization of USP15, a novel human ubiquitin-specific protease related to the UNP oncoprotein, and a systematic nomenclature for human ubiquitin-specific proteases. Genomics 59: 264-274.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 604731. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Angelats, C., et al. 2003. Isolation and characterization of the mouse ubiquitin-specific protease USP15. Mamm. Genome 14: 31-46.
5. Hetfeld, B.K., et al. 2005. The zinc finger of the CSN-associated deubiquitinating enzyme USP15 is essential to rescue the E3 ligase Rbx1. Curr. Biol. 15: 1217-1221.

CHROMOSOMAL LOCATION

Genetic locus: Usp15 (mouse) mapping to 10 D2.

PRODUCT

USP15 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 USP15 shRNA Plasmid (m): sc-76820-SH and USP15 shRNA (m) Lentiviral Particles: sc-76820-V as alternate gene silencing products.

For independent verification of USP15 (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-76820A, sc-76820B and sc-76820C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

USP15 siRNA (m) is recommended for the inhibition of USP15 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

USP15 (2D5): sc-100629 is recommended as a control antibody for monitoring of USP15 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 USP15 gene expression knockdown using RT-PCR Primer: USP15 (m)-PR: sc-76820-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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