SANTA CRUZ BIOTECHNOLOGY, INC.

USP5 siRNA (h): sc-76869

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. USP5 (ubiquitin specific peptidase 5), also known as ISOT (isopeptidase T), is a 858 amino acid zinc-binding deubiquitinating enzyme that participates in the Ub pathway. A member of the peptidase C19 family, the catalytic activity of USP5 involves a combination of the ubiquitin carboxyl-terminal thiolester and water to produce ubiquitin and a thiol. USP5 contains two UBA domains and one UBP-type zinc finger. USP5 is responsible for disassembling unanchored polyubiquitin chains in the cell.

REFERENCES

1. Stein, R.L., et al. 1995. Kinetic studies of isopeptidase T: modulation of peptidase activity by ubiquitin. Biochemistry 34: 12616-12623.
2. Wilkinson, K.D., et al. 1995. Metabolism of the polyubiquitin degradation signal: structure, mechanism, and role of isopeptidase T. Biochemistry 34: 14535-14546.
3. Melandri, F., et al. 1996. Kinetic studies on the inhibition of isopeptidase T by ubiquitin aldehyde. Biochemistry 35: 12893-12900.
4. Engidawork, E., et al. 2001. Selective upregulation of the ubiquitin-proteasome proteolytic pathway proteins, proteasome ζ chain and isopeptidase T in fetal Down syndrome. J. Neural Transm. Suppl. 61: 117-130.

CHROMOSOMAL LOCATION

Genetic locus: USP5 (human) mapping to 12p13.31.

PRODUCT

USP5 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 USP5 shRNA Plasmid (h): sc-76869-SH and USP5 shRNA (h) Lentiviral Particles: sc-76869-V as alternate gene silencing products.

For independent verification of USP5 (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-76869A, sc-76869B and sc-76869C.

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

USP5 siRNA (h) is recommended for the inhibition of USP5 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

USP5 (C-11): sc-390943 is recommended as a control antibody for monitoring of USP5 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 USP5 gene expression knockdown using RT-PCR Primer: USP5 (h)-PR: sc-76869-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chen, Y., et al. 2016. Wnt-induced deubiquitination FoxM1 ensures nucleus β-catenin transactivation. EMBO J. 35: 668-684.
2. Kim, S., et al. 2019. WP1130 enhances TRAIL-induced apoptosis through USP9X-dependent miR-708-mediated downregulation of c-FLIP. Cancers 11: 344.
3. Vashistha, V., et al. 2020. Depleting deubiquitinating enzymes promotes apoptosis in glioma cell line via RNA binding proteins SF2/ASF1. Biochem. Biophys. Rep. 24: 100846.
4. Ramachandran, A., et al. 2021. Deubiquitination and activation of the NLRP3 inflammasome by UCHL5 in HCV-infected cells. Microbiol. Spectr. E-published.

RESEARCH USE

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