SANTA CRUZ BIOTECHNOLOGY, INC.

UBPY siRNA (m): sc-76796

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. UBPY, also known as USP8 (ubiquitin carboxyl-terminal hydrolase 8) or KIAA0055, is a 1,118 amino acid protein that contains one rhodanese domain and exists in a ternary complex with OTUB1 and GRAIL. Functioning as a hydrolase, UBPY catalyzes the removal of ubiquitin from ubiquitin-conjugated proteins and plays an important role in protein turnover, cellular proliferation and T-cell anergy. The gene encoding UBPY maps to human chromosome 15, which houses over 700 genes and comprises nearly 3% of the human genome.

REFERENCES

1. Naviglio, S., et al. 1998. UBPY: a growth-regulated human ubiquitin isopeptidase. EMBO J. 17: 3241-3250.
2. Kato, M., et al. 2000. A deubiquitinating enzyme UBPY interacts with the Src homology 3 domain of Hrs-binding protein via a novel binding motif PX(V/I)(D/N)RXXKP. J. Biol. Chem. 275: 37481-37487.
3. Gnesutta, N., et al. 2001. Cloning and characterization of mouse UBPy, a deubiquitinating enzyme that interacts with the ras guanine nucleotide exchange factor CDC25(Mm)/Ras-GRF1. J. Biol. Chem. 276: 39448-39454.
4. Berruti, G., et al. 2005. The deubiquitinating enzyme mUBPy interacts with the sperm-specific molecular chaperone MSJ-1: the relation with the proteasome, acrosome, and centrosome in mouse male germ cells. Biol. Reprod. 72: 14-21.

CHROMOSOMAL LOCATION

Genetic locus: Usp8 (mouse) mapping to 2 F1.

PRODUCT

UBPY 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBPY shRNA Plasmid (m): sc-76796-SH and UBPY shRNA (m) Lentiviral Particles: sc-76796-V as alternate gene silencing products.

For independent verification of UBPY (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-76796A, sc-76796B and sc-76796C.

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

UBPY siRNA (m) is recommended for the inhibition of UBPY 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 µM in 60 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

UBPY (E-1): sc-376130 is recommended as a control antibody for monitoring of UBPY 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 UBPY gene expression knockdown using RT-PCR Primer: UBPY (m)-PR: sc-76796-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. Zhang, Y., et al. 2018. Effect of deubiquitinase USP8 on hypoxia/reoxygenation-induced inflammation by deubiquitination of TAK1 in renal tubular epithelial cells. Int. J. Mol. Med. 42: 3467-3476.
2. Sun, Y., et al. 2020. Exogenous H_2S promoted USP8 sulfhydration to regulate mitophagy in the hearts of db/db mice. Aging Dis. 11: 269-285.

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.