SANTA CRUZ BIOTECHNOLOGY, INC.

USP18 siRNA (h): sc-60865

BACKGROUND

The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. A wide range of enzymes facilitate the proteolytic Ub pathway, including the ubiquitin specific peptidase, USP18 (also designated interferon-stimulated gene 43 (ISG43), ISG15-specific-processing protease, Ubl carboxyl-terminal hydrolase 18 and Ubp43). USP18, a member of the peptidase C19 family, maintains a critical cellular balance of ISG15-conjugated proteins in stressed and healthy organisms. It can efficiently cleave ISG15 fusions including native ISG15 conjugates linked by isopeptide bonds. In mice, deletion of the USP18 gene leads to a large increase of ISG15 conjugates in tissues. USP18 expression is negatively regulated by RNase-L and induced by interferon.

REFERENCES

1. Liu, L.Q., et al. 1999. A novel ubiquitin-specific protease, UBP4 cloned from leukemia fusion protein AML1-ETO-expressing mice, functions in hematopoietic cell differentiation. Mol. Cell. Biol. 19: 3029-3038.
2. Schwer, H., et al. 2000. Cloning and characterization of a novel human ubiquitin-specific protease, a homologue of murine UBP43 (USP18). Genomics 65: 44-52.
3. Li, X.L., et al. 2000. RNase-L-dependent destabilization of interferon-induced mRNAs. A role for the 2-system in attenuation of the interferon response. J. Biol. Chem. 275: 8880-8888.
4. Malakhov, M.P., et al. 2002. UBP43 (USP18) specifically removes ISG15 from conjugated proteins. J. Biol. Chem. 277: 9976-9981.

CHROMOSOMAL LOCATION

Genetic locus: USP18 (human) mapping to 22q11.21.

PRODUCT

USP18 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 USP18 shRNA Plasmid (h): sc-60865-SH and USP18 shRNA (h) Lentiviral Particles: sc-60865-V as alternate gene silencing products.

For independent verification of USP18 (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-60865A, sc-60865B and sc-60865C.

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

USP18 siRNA (h) is recommended for the inhibition of USP18 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

USP18 (E-5): sc-374064 is recommended as a control antibody for monitoring of USP18 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 USP18 gene expression knockdown using RT-PCR Primer: USP18 (h)-PR: sc-60865-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. Bagchi, P., et al. 2012. Identification of common human host genes involved in pathogenesis of different rotavirus strains: an attempt to recognize probable antiviral targets. Virus Res. 169: 144-153.
2. Sung, P.S., et al. 2017. IFN-λ4 potently blocks IFN-α signalling by ISG15 and USP18 in hepatitis C virus infection. Sci. Rep. 7: 3821.

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.