

USP29 siRNA (h): sc-76833

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. USP29 (ubiquitin specific peptidase 29), also known as HOM-TES-84/86, is a 922 amino acid deubiquitinating enzyme that participates in the Ub pathway. A member of the peptidase C19 family, the catalytic activity of USP29 involves a combination of the ubiquitin carboxyl-terminal thiolester and water to produce ubiquitin and a thiol. USP29 contains a cys box and a his box, which are characteristic of type-2 ubiquitin C-terminal hydrolases.

REFERENCES

1. D'Andrea, A., et al. 1998. Deubiquitinating enzymes: a new class of biological regulators. *Crit. Rev. Biochem. Mol. Biol.* 33: 337-352.
2. Chung, C.H., et al. 1999. Deubiquitinating enzymes: their diversity and emerging roles. *Biochem. Biophys. Res. Commun.* 266: 633-640.
3. Kim, J., et al. 2000. Discovery of a novel, paternally expressed ubiquitin-specific processing protease gene through comparative analysis of an imprinted region of mouse chromosome 7 and human chromosome 19q13.4. *Genome Res.* 10: 1138-1147.
4. Kim, J., et al. 2001. Imprinting and evolution of two Krüppel-type zinc-finger genes, ZIM3 and ZNF264, located in the PEG3/USP29 imprinted domain. *Genomics* 77: 91-98.
5. Online Mendelian Inheritance in Man, OMIM™. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 609546. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Kim, J., et al. 2007. Genomic organization and imprinting of the Peg3 domain in bovine. *Genomics* 90: 85-92.

CHROMOSOMAL LOCATION

Genetic locus: USP29 (human) mapping to 19q13.43.

PRODUCT

USP29 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see USP29 shRNA Plasmid (h): sc-76833-SH and USP29 shRNA (h) Lentiviral Particles: sc-76833-V as alternate gene silencing products.

For independent verification of USP29 (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-76833A, sc-76833B and sc-76833C.

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

USP29 siRNA (h) is recommended for the inhibition of USP29 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 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

USP29 (1A8): sc-517145 is recommended as a control antibody for monitoring of USP29 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 USP29 gene expression knockdown using RT-PCR Primer: USP29 (h)-PR: sc-76833-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. Jo, A., et al. 2019. Deubiquitinase USP29 governs MYBBP1A in the brains of Parkinson's disease patients. *J. Clin. Med.* pii: E52.

RESEARCH USE

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