

USP32 siRNA (m): sc-106679

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. USP32 (ubiquitin specific peptidase 32), also known as NY-REN-60, is a 1,604 amino acid protein that contains one DUSP domain and three EF-hand calcium binding domains. Localized to membranes in a lipid-anchored fashion and expressed in all normal tissues, USP32 catalyzes the conversion of a ubiquitin C-terminal thioester to a free ubiquitin and a thiol, a reaction that may influence several cellular processes.

REFERENCES

1. Wilkinson, K.D. 1997. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J.* 11: 1245-1256.
2. Scanlan, M.J., Gordan, J.D., Williamson, B., Stockert, E., Bander, N.H., Jongeneel, V., Gure, A.O., Jäger, D., Jäger, E., Knuth, A., Chen, Y.T. and Old, L.J. 1999. Antigens recognized by autologous antibody in patients with renal-cell carcinoma. *Int. J. Cancer* 83: 456-464.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607740. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Puente, X.S., Sánchez, L.M., Overall, C.M. and López-Otín, C. 2003. Human and mouse proteases: a comparative genomic approach. *Nat. Rev. Genet.* 4: 544-558.
5. Paulding, C.A., Ruvoletto, M. and Haber, D.A. 2003. The Tre2 (USP6) oncogene is a hominoid-specific gene. *Proc. Natl. Acad. Sci. USA* 100: 2507-2511.
6. Quesada, V., Díaz-Perales, A., Gutiérrez-Fernández, A., Garabaya, C., Cal, S. and López-Otín, C. 2004. Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. *Biochem. Biophys. Res. Commun.* 314: 54-62.

CHROMOSOMAL LOCATION

Genetic locus: *Usp32* (mouse) mapping to 11 C.

PRODUCT

USP32 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 USP32 shRNA Plasmid (m): sc-106679-SH and USP32 shRNA (m) Lentiviral Particles: sc-106679-V as alternate gene silencing products.

For independent verification of USP32 (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-106679A, sc-106679B and sc-106679C.

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

USP32 siRNA (m) is recommended for the inhibition of USP32 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

USP32 (A-10): sc-374465 is recommended as a control antibody for monitoring of USP32 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 USP32 gene expression knockdown using RT-PCR Primer: USP32 (m)-PR: sc-106679-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.