SANTA CRUZ BIOTECHNOLOGY, INC.

USP40 siRNA (h): sc-94351

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. USP40 (ubiquitin specific peptidase 40), also known as ubiquitin thioesterase 40, deubiquitinating enzyme 40 or ubiquitin carboxyl-terminal hydrolase 40, is a 1,235 amino acid protein that belongs to a large family of cysteine proteases that function as deubiquitinating enzymes. Broadly expressed, USP40 catalyzes the conversion of a ubiquitin C-terminal thioester to a free ubiquitin and a thiol, a reaction that may influence several cellular processes. Existing as two isoforms due to alternative splicing events, USP40 is considered a novel target for late-onset Parkinson disease.

REFERENCES

1. Chung, C.H. and Baek, S.H. 1999. Deubiquitinating enzymes: their diversity and emerging roles. Biochem. Biophys. Res. Commun. 266: 633-640.
2. Southan, C. 2001. A genomic perspective on human proteases. FEBS Lett. 498: 214-218.
3. Puente, X.S., et al. 2003. Human and mouse proteases: a comparative genomic approach. Nat. Rev. Genet. 4: 544-558.
4. Quesada, V., et al. 2004. Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. Biochem. Biophys. Res. Commun. 314: 54-62.
5. Li, Y., et al. 2006. Genetic evidence for ubiquitin-specific proteases USP24 and USP40 as candidate genes for late-onset Parkinson disease. Hum. Mutat. 27: 1017-1023.
6. Deng, S., et al. 2007. Overexpression of genes and proteins of ubiquitin specific peptidases (USPs) and proteasome subunits (PSs) in breast cancer tissue observed by the methods of RFDD-PCR and proteomics. Breast Cancer Res. Treat. 104: 21-30.

CHROMOSOMAL LOCATION

Genetic locus: USP40 (human) mapping to 2q37.1.

PRODUCT

USP40 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 USP40 shRNA Plasmid (h): sc-94351-SH and USP40 shRNA (h) Lentiviral Particles: sc-94351-V as alternate gene silencing products.

For independent verification of USP40 (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-94351A, sc-94351B and sc-94351C.

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 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

USP40 siRNA (h) is recommended for the inhibition of USP40 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

USP40 (G-8): sc-514248 is recommended as a control antibody for monitoring of USP40 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 USP40 gene expression knockdown using RT-PCR Primer: USP40 (h)-PR: sc-94351-PR (20 µl). 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.