

# USP4 siRNA (m): sc-76852

## 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. USP4 (ubiquitin-specific-processing protease 4), also known as ubiquitin carboxyl-terminal hydrolase 4, UNP or UNPH (ubiquitous nuclear protein homolog), is a 963 amino acid nucleocytoplasmic protein that belongs to the peptidase C19 family. USP4 binds to the C-terminus of Adenosine A<sub>2A</sub>-R, a G<sub>s</sub>-coupled receptor, and enhances cell surface expression of the functionally active receptor. USP4 contains one DUSP domain and exists as two isoforms due to alternative splicing.

## REFERENCES

1. Frederick, A., et al. 1998. The human UNP locus at 3p21.31 encodes two tissue-selective, cytoplasmic isoforms with deubiquitinating activity that have reduced expression in small cell lung carcinoma cell lines. *Oncogene* 16: 153-165.
2. D'Andrea, A. and Pellman, D. 1998. Deubiquitinating enzymes: a new class of biological regulators. *Crit. Rev. Biochem. Mol. Biol.* 33: 337-352.
3. DeSalle, L.M., et al. 2001. The de-ubiquitinating enzyme UNP interacts with the retinoblastoma protein. *Oncogene* 20: 5538-5542.
4. Soboleva, T.A., et al. 2005. Nuclear-cytoplasmic shuttling of the oncogenic mouse UNP/USP4 deubiquitylating enzyme. *J. Biol. Chem.* 280: 745-752.
5. Wada, K. and Kamitani, T. 2006. UnpEL/USP4 is ubiquitinated by Ro52 and deubiquitinated by itself. *Biochem. Biophys. Res. Commun.* 342: 253-258.
6. Toews, M.L. 2006. Adenosine receptors find a new partner and move out. *Mol. Pharmacol.* 69: 1075-1078.
7. Mijoljevic, T., et al. 2006. The ubiquitin-specific protease USP4 regulates the cell surface level of the A<sub>2A</sub> receptor. *Mol. Pharmacol.* 69: 1083-1094.
8. Gsandtner, I. and Freissmuth, M. 2006. A tail of two signals: the C terminus of the A<sub>2A</sub>-adenosine receptor recruits alternative signaling pathways. *Mol. Pharmacol.* 70: 447-449.

## CHROMOSOMAL LOCATION

Genetic locus: Usp4 (mouse) mapping to 9 F2.

## PRODUCT

USP4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see USP4 shRNA Plasmid (m): sc-76852-SH and USP4 shRNA (m) Lentiviral Particles: sc-76852-V as alternate gene silencing products.

For independent verification of USP4 (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-76852A, sc-76852B and sc-76852C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

USP4 siRNA (m) is recommended for the inhibition of USP4 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  $\mu$ M in 66  $\mu$ 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

USP4 (H-3): sc-376000 is recommended as a control antibody for monitoring of USP4 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor USP4 gene expression knockdown using RT-PCR Primer: USP4 (m)-PR: sc-76852-PR (20  $\mu$ 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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.