

# USP33 siRNA (h): sc-76839

## 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. USP33 (ubiquitin specific peptidase 33), also known as VDU1 (VHL-interacting deubiquitinating enzyme 1), is a widely expressed 942 amino acid protein that belongs to the peptidase C19 family of proteins. Containing two DUSP domains and a UBP-type zinc finger, USP33 functions as deubiquitinating enzyme that cleaves ubiquitin residues from both ubiquitinated proteins and ubiquitin-fused precursors, thereby saving these proteins from proteasomal degradation. In addition, USP33 binds VHL and can be ubiquitinated for degradation in a VHL-dependent manner. Three isoforms of USP33 are expressed due to alternative splicing events.

## REFERENCES

1. Li, Z., et al. 2002. Identification of a deubiquitinating enzyme subfamily as substrates of the von Hippel-Lindau tumor suppressor. *Biochem. Biophys. Res. Commun.* 294: 700-709.
2. Li, Z., et al. 2002. Ubiquitination of a novel deubiquitinating enzyme requires direct binding to von Hippel-Lindau tumor suppressor protein. *J. Biol. Chem.* 277: 4656-4662.
3. Curcio-Morelli, C., et al. 2003. Deubiquitination of type 2 iodothyronine deiodinase by von Hippel-Lindau protein-interacting deubiquitinating enzymes regulates thyroid hormone activation. *J. Clin. Invest.* 112: 189-196.
4. Puente, X.S., et al. 2003. Human and mouse proteases: a comparative genomic approach. *Nat. Rev. Genet.* 4: 544-558.
5. Bianco, A.C. 2004. Triplets! Unexpected structural similarity among the three enzymes that catalyze initiation and termination of thyroid hormone effects. *Arq. Bras. Endocrinol. Metabol.* 48: 16-24.

## CHROMOSOMAL LOCATION

Genetic locus: USP33 (human) mapping to 1p31.1.

## PRODUCT

USP33 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see USP33 shRNA Plasmid (h): sc-76839-SH and USP33 shRNA (h) Lentiviral Particles: sc-76839-V as alternate gene silencing products.

For independent verification of USP33 (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-76839A, sc-76839B and sc-76839C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ 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

USP33 siRNA (h) is recommended for the inhibition of USP33 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  $\mu$ 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

USP33 (1D7): sc-100632 is recommended as a control antibody for monitoring of USP33 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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor USP33 gene expression knockdown using RT-PCR Primer: USP33 (h)-PR: sc-76839-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.