

# USP9X siRNA (m): sc-63198

## 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. USP9X (ubiquitin specific peptidase 9, X-linked), also known as FAF or DFFRX, is a 2,547 amino acid member of the peptidase C19 family of ubiquitin proteases. Expressed ubiquitously in both fetal and adult tissue, USP9X is involved in the processing of ubiquitin precursors and ubiquitinated proteins, thereby preventing degradation and regulating protein turnover. Defects in the gene encoding USP9X are implicated in Turner syndrome, a condition in which oocytes fail to proliferate and develop, leading to the degeneration of the developing ovary. Multiple isoforms encoding long and short transcripts exist due to alternative splicing events.

## REFERENCES

1. Brown, G.M., et al. 1998. Characterisation of the coding sequence and fine mapping of the human DFFRY gene and comparative expression analysis and mapping to the Sxrb interval of the mouse Y chromosome of the Dffry gene. *Hum. Mol. Genet.* 7: 97-107.
2. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 300072. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

## CHROMOSOMAL LOCATION

Genetic locus: Usp9x (mouse) mapping to X A1.1.

## PRODUCT

USP9X 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 USP9X shRNA Plasmid (m): sc-63198-SH and USP9X shRNA (m) Lentiviral Particles: sc-63198-V as alternate gene silencing products.

For independent verification of USP9X (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-63198A, sc-63198B and sc-63198C.

## 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.

## RESEARCH USE

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

## APPLICATIONS

USP9X siRNA (m) is recommended for the inhibition of USP9X 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

USP9X/Y (E-12): sc-365353 is recommended as a control antibody for monitoring of USP9X 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 USP9X gene expression knockdown using RT-PCR Primer: USP9X (m)-PR: sc-63198-PR (20  $\mu$ l, 600 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Wang, P., et al. 2012. Loss of AMP-activated protein kinase- $\alpha$ 2 impairs the Insulin-sensitizing effect of calorie restriction in skeletal muscle. *Diabetes* 61: 1051-1061.

## PROTOCOLS

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