

## ITM1 siRNA (m): sc-146310

### BACKGROUND

ITM1 (integral membrane protein 1), also known as TMC (transmembrane protein TMC) or STT3A (STT3, subunit of the oligosaccharyltransferase complex, homolog A), is a member of the STT3 family of proteins. Predominantly expressed in liver, pancreas, muscle, placenta and skin fibroblasts, ITM1 is a multi-pass membrane protein that localizes to the membrane of the endoplasmic reticulum (ER). ITM1 is one of two multicellular eukaryotic homologs of the *S. cerevisiae* protein Stt3, an essential component of the yeast OST (oligosaccharyltransferase) complex. Both homologs (ITM1 and SIMP) are glycosylated and function as the catalytic component of the mammalian OST complex which is responsible for catalyzing the transfer of a high mannose oligosaccharide to an asparagine residue in nascent proteins that enter the lumen of the ER. Using lipid-linked oligosaccharides as donors, the OST complex specifically transfers the oligosaccharide to the asparagine residue in an Asn-X-Ser/Thr consensus motif (X is any amino acid excluding proline). Compared with SIMP, ITM1 is less active but also more selective in terms of substrates.

### REFERENCES

1. Lissy, N.A., et al. 1996. Isolation, characterization, and mapping to human chromosome 11q24-25 of a cDNA encoding a highly conserved putative transmembrane protein, TMC. *Biochim. Biophys. Acta* 1306: 137-141.
2. Hong, G., et al. 1996. Molecular cloning of a highly conserved mouse and human integral membrane protein (Itm1) and genetic mapping to mouse chromosome 9. *Genomics* 31: 295-300.
3. Van Hul, W., et al. 1996. Assignment of the human integral transmembrane protein 1 gene (ITM1) to human chromosome band 11q23.3 by *in situ* hybridization and YAC mapping. *Cytogenet. Cell Genet.* 74: 218-219.
4. Yoshida, S., et al. 1999. *Schizosaccharomyces pombe* stt3<sup>+</sup> is a functional homologue of *Saccharomyces cerevisiae* STT3 which regulates oligosaccharyltransferase activity. *Yeast* 15: 497-505.
5. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 601134. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

### CHROMOSOMAL LOCATION

Genetic locus: Stt3a (mouse) mapping to 9 A4.

### PRODUCT

ITM1 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 ITM1 shRNA Plasmid (m): sc-146310-SH and ITM1 shRNA (m) Lentiviral Particles: sc-146310-V as alternate gene silencing products.

For independent verification of ITM1 (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-146310A, sc-146310B and sc-146310C.

### 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

ITM1 siRNA (m) is recommended for the inhibition of ITM1 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

ITM1 (A-2): sc-390227 is recommended as a control antibody for monitoring of ITM1 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 ITM1 gene expression knockdown using RT-PCR Primer: ITM1 (m)-PR: sc-146310-PR (20  $\mu$ l, 371 bp). 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.