

## TMEM66 siRNA (h): sc-77778

### BACKGROUND

Calcium is considered an essential second messenger in the regulation of many important biological processes, including cell proliferation, differentiation, migration and apoptosis. One major pathway for calcium entry and signaling in nonexcitable cells is through store-operated calcium channels (SOCs). Activation of SOCs is triggered by depletion of intracellular  $\text{Ca}^{2+}$  and mediated by specific  $\text{Ca}^{2+}$  sensor surface receptors of the endoplasmic reticulum. TMEM66 (transmembrane protein 66), also known as SARAF (store-operated calcium entry-associated regulatory factor), XTP3, FOAP-7 or HSPC035 is a 339 amino acid single-pass type I membrane protein that localizes to the endoplasmic reticulum. Highly expressed in macrophages, TMEM66 acts as a negative regulator of store-operated  $\text{Ca}^{2+}$  entry (SOCE), protecting cells from excessive uptake of  $\text{Ca}^{2+}$ . Elevation of cytosolic  $\text{Ca}^{2+}$  induces TMEM66, promoting slow inactivation of STIM1 or STIM2-dependent SOCE activity, leveling  $\text{Ca}^{2+}$  uptake. TMEM66 exists as two alternatively spliced isoforms and is encoded by a gene located on human chromosome 8p12.

### REFERENCES

1. Mannherz, O., et al. 2006. Functional screening for proapoptotic genes by reverse transfection cell array technology. *Genomics* 87: 665-672.
2. Palty, R., et al. 2012. SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. *Cell* 149: 425-438.
3. Lodola, F., et al. 2012. Store-operated  $\text{Ca}^{2+}$  entry is remodelled and controls *in vitro* angiogenesis in endothelial progenitor cells isolated from tumoral patients. *PLoS ONE* 7: e42541.
4. Zhang, J., et al. 2013. Inhibition of store-operated  $\text{Ca}^{2+}$  entry suppresses EGF-induced migration and eliminates extravasation from vasculature in nasopharyngeal carcinoma cell. *Cancer Lett.* 336: 390-397.
5. Jha, A., et al. 2013. The STIM1 CTID domain determines access of SARAF to SOAR to regulate Orai1 channel function. *J. Cell Biol.* 202: 71-79.
6. Taha, S., et al. 2015. Differential upregulation of the hypothetical transmembrane protein 66 (TMEM66) in multiple sclerosis patients with potential inflammatory response. *Biomed. Rep.* 3: 98-104.
7. Xie, J., et al. 2015. SOCE and cancer: Recent progress and new perspectives. *Int. J. Cancer* 138: 2067-2077.
8. Prakriya, M. and Lewis, R.S. 2015. Store-operated calcium channels. *Physiol. Rev.* 95: 1383-1436.

### CHROMOSOMAL LOCATION

Genetic locus: SARAF (human) mapping to 8p12.

### 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.

### PRODUCT

TMEM66 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  $\mu\text{M}$  solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TMEM66 shRNA Plasmid (h): sc-77778-SH and TMEM66 shRNA (h) Lentiviral Particles: sc-77778-V as alternate gene silencing products.

For independent verification of TMEM66 (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-77778A, sc-77778B and sc-77778C.

### STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}\text{C}$  with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}\text{C}$ , avoid contact with RNAses and repeated freeze thaw cycles.

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

### APPLICATIONS

TMEM66 siRNA (h) is recommended for the inhibition of TMEM66 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  $\mu\text{M}$  in 66  $\mu\text{l}$ . 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.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TMEM66 gene expression knockdown using RT-PCR Primer: TMEM66 (h)-PR: sc-77778-PR (20  $\mu\text{l}$ ). Annealing temperature for the primers should be  $55-60^{\circ}\text{C}$  and the extension temperature should be  $68-72^{\circ}\text{C}$ .