

## EDG-6 siRNA (h): sc-43747

### BACKGROUND

The EDG (endothelial differentiation gene) family of G protein-coupled receptors consists of eight family members that bind lysophospholipid (LPL) mediators, including sphingosine-1-phosphate (SPP) and lysophosphatidic acid (LPA). EDG-1, EDG-3, EDG-5 (also designated H218 and AGR16) and EDG-8 bind SPP with high affinity. EDG-6 is a low affinity receptor for SPP. LPA preferentially binds to EDG-2, EDG-4 and EDG-7. The EDG receptors couple to multiple G proteins to signal through Ras, MAP kinase, Rho, Phospholipase C or other tyrosine kinases, which lead to cell survival, growth, migration and differentiation. EDG-1 signals through G<sub>i</sub> proteins to activate Akt and is expressed in glioma cells. EDG-2 is expressed in brain, especially in white matter tract regions, while EDG-3 is expressed in cardiovascular tissue and in cerebellum. EDG-4 is highly expressed on leukocytes and brain, and EDG-5 has wide tissue distribution, including cardiovascular tissue and brain. EDG-6, which is expressed in lymphoid and hematopoietic tissues and in lung, signals through G<sub>i/o</sub> proteins, which activate growth related pathways.

### REFERENCES

- Goetzl, E.J., et al. 1999. A subfamily of G protein-coupled cellular receptors for lysophospholipids and lysosphingolipids. *Adv. Exp. Med. Biol.* 469: 259-264.
- Van Brocklyn, J.R., et al. 2000. Sphingosine 1-phosphate is a ligand for the G protein-coupled receptor EDG-6. *Blood* 95: 2624-2629.
- Sato, K., et al. 2000. Differential roles of EDG-1 and EDG-5, sphingosine 1-phosphate receptors, in the signaling pathways in C6 glioma cells. *Brain Res. Mol. Brain Res.* 85: 151-160.
- Pyne, S., et al. 2000. Sphingosine 1-phosphate signalling in mammalian cells. *Biochem. J.* 349: 385-402.
- Zheng, Y., et al. 2001. Lysophosphatidic acid receptor-selective effects on Jurkat T cell migration through a matrigel model basement membrane. *J. Immunol.* 166: 2317-2322.
- Morales-Ruiz, M., et al. 2001. Sphingosine-1-phosphate activates Akt, nitric oxide production and chemotaxis through a G<sub>i</sub>-protein/phosphoinositide 3-kinase pathway in endothelial cells. *J. Biol. Chem.* 276: 19672-19677.

### CHROMOSOMAL LOCATION

Genetic locus: S1PR4 (human) mapping to 19p13.3.

### PRODUCT

EDG-6 siRNA (h) is a pool of 2 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 EDG-6 shRNA Plasmid (h): sc-43747-SH and EDG-6 shRNA (h) Lentiviral Particles: sc-43747-V as alternate gene silencing products.

For independent verification of EDG-6 (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-43747A and sc-43747B.

### 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

EDG-6 siRNA (h) is recommended for the inhibition of EDG-6 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

EDG-6 (1): sc-65216 is recommended as a control antibody for monitoring of EDG-6 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).

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EDG-6 gene expression knockdown using RT-PCR Primer: EDG-6 (h)-PR: sc-43747-PR (20  $\mu$ l, 459 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

- Lin, C.I., et al. 2007. Sphingosine 1-phosphate regulates inflammation-related genes in human endothelial cells through S1P1 and S1P3. *Biochem. Biophys. Res. Commun.* 355: 895-901.
- Long, J.S., et al. 2010. Sphingosine 1-phosphate receptor 4 uses HER2 (ERBB2) to regulate extracellular signal regulated kinase-1/2 in MDA-MB-453 breast cancer cells. *J. Biol. Chem.* 285: 35957-35966.

### 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.