

SERGEF siRNA (m): sc-153345

BACKGROUND

SERGEF (secretion-regulating guanine nucleotide exchange factor), also called DelGEF (deafness locus-associated putative guanine nucleotide exchange factor), is a guanine nucleotide exchange factor which is thought to be involved in secretion pathways. SERGEF associates with Sec5, a protein required for secretion, in a magnesium-dependent manner and is stimulated by the presence of deoxycytidine triphosphate (dCTP) or guanosine triphosphate (GTP). A homolog of RanGEF, SERGEF is localized to the nucleus and cytoplasm and is expressed throughout the body with high expression observed in the brain, placenta and skeletal muscle. SERGEF is a 458 amino acid protein which, upon DNA damage, is phosphorylated by Atm or ATR. Two isoforms exist due to alternative splicing.

REFERENCES

- Uhlmann, J., Wiemann, S. and Ponstingl, H. 1999. DelGEF, an RCC1-related protein encoded by a gene on chromosome 11p.critical for two forms of hereditary deafness. *FEBS Lett.* 460: 153-160.
- Sjölander, M., Uhlmann, J. and Ponstingl, H. 2002. DelGEF, a homologue of the Ran guanine nucleotide exchange factor RanGEF, binds to the exocyst component Sec5 and modulates secretion. *FEBS Lett.* 532: 211-215.
- Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606051. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Mott, H.R., Nietlispach, D., Hopkins, L.J., Mirey, G., Camonis, J.H. and Owen, D. 2003. Structure of the GTPase-binding domain of Sec5 and elucidation of its Ral binding site. *J. Biol. Chem.* 278: 17053-17059.
- Sjölander, M., Uhlmann, J. and Ponstingl, H. 2004. Characterisation of an evolutionary conserved protein interacting with the putative guanine nucleotide exchange factor DelGEF and modulating secretion. *Exp. Cell Res.* 294: 68-76.

CHROMOSOMAL LOCATION

Genetic locus: *Sergef* (mouse) mapping to 7 B4.

PRODUCT

SERGEF 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SERGEF shRNA Plasmid (m): sc-153345-SH and SERGEF shRNA (m) Lentiviral Particles: sc-153345-V as alternate gene silencing products.

For independent verification of SERGEF (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-153345A, sc-153345B and sc-153345C.

PROTOCOLS

See our web site at 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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SERGEF siRNA (m) is recommended for the inhibition of SERGEF 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 μ M in 66 μ 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

SERGEF (H-12): sc-515188 is recommended as a control antibody for monitoring of SERGEF 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 SERGEF gene expression knockdown using RT-PCR Primer: SERGEF (m)-PR: sc-153345-PR (20 μ 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.