

Elmo3 siRNA (m): sc-44582

BACKGROUND

Elmo (engulfment and cell motility) proteins share similarity to *C. elegans* CED-12. The *C. elegans* genes *ced-2*, *ced-5*, *ced-10*, and *ced-12* and their mammalian homologs CRKII, DOCK1, RAC1 and ELMO mediate cytoskeletal rearrangements during phagocytosis of apoptotic cells and cell motility. Elmo1 associates with DOCK 180 and may influence phagocytosis and effect cell shape changes. Src family kinase mediated tyrosine phosphorylation of Elmo1 influences signaling through Elmo1/Crk/DOCK 180 pathways. Elmo2 interacts directly with Rho G in a GTP-dependent manner and forms a ternary complex with DOCK 180 to induce activation of Rac 1. The Rho G-Elmo2-DOCK 180 pathway is required for activation of Rac 1 and cell spreading mediated by integrin, as well as for neurite outgrowth induced by nerve growth factor. Elmo3 acts in association with DOCK 180 and Crk II and may be required in complex with DOCK 180 to activate Rac/Rho small GTPases.

REFERENCES

1. Gumienny, T.L., et al. 2001. CED-12/Elmo, a novel member of the Crk II/DOCK 180/Rac pathway, is required for phagocytosis and cell migration. *Cell* 107: 27-41.
2. Brugnera, E., et al. 2002. Unconventional Rac-GEF activity is mediated through the DOCK 180-Elmo complex. *Nat. Cell Biol.* 4: 574-582.
3. Katoh, H., et al. 2003. Rho G activates Rac 1 by direct interaction with the DOCK 180-binding protein Elmo. *Nature* 424: 461-464.
4. Sanui, T., et al. 2003. DOCK2 regulates Rac activation and cytoskeletal reorganization through interaction with Elmo1. *Blood* 102: 2948-2950.
5. Lu, M., et al. 2004. PH domain of Elmo functions *in trans* to regulate Rac activation via DOCK 180. *Nat. Struct. Mol. Biol.* 11: 756-762.
6. deBakker, C.D., et al. 2004. Phagocytosis of apoptotic cells is regulated by a UNC-73/TRIO-MIG-2/Rho G signaling module and armadillo repeats of CED-12/Elmo. *Curr. Biol.* 14: 2208-2216.
7. Yokoyama, N., et al. 2005. Identification of tyrosine residues on Elmo1 that are phosphorylated by the Src-family kinase Hck. *Biochemistry* 44: 8841-8849.

CHROMOSOMAL LOCATION

Genetic locus: Elmo3 (mouse) mapping to 8 D3.

PRODUCT

Elmo3 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 Elmo3 shRNA Plasmid (m): sc-44582-SH and Elmo3 shRNA (m) Lentiviral Particles: sc-44582-V as alternate gene silencing products.

For independent verification of Elmo3 (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-44582A, sc-44582B and sc-44582C.

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

Elmo3 siRNA (m) is recommended for the inhibition of Elmo3 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

Elmo3 (E-4): sc-166364 is recommended as a control antibody for monitoring of Elmo3 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 Elmo3 gene expression knockdown using RT-PCR Primer: Elmo3 (m)-PR: sc-44582-PR (20 μ l, 528 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 for detailed protocols and support products.