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# Elmo3 shRNA (m) Lentiviral Particles: sc-44582-V

## 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 Crk II, Dock 1, Rac 1 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

- 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.
- Brugnera, E., et al. 2002. Unconventional Rac-GEF activity is mediated through the DOCK 180-Elmo complex. *Nat. Cell Biol.* 4: 574-582.
- Katoh, H., et al. 2003. Rho G activates Rac 1 by direct interaction with the DOCK 180-binding protein Elmo. *Nature* 424: 461-464.
- Sanui, T., et al. 2003. DOCK2 regulates Rac activation and cytoskeletal reorganization through interaction with Elmo1. *Blood* 102: 2948-2950.
- 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.

## CHROMOSOMAL LOCATION

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

## PRODUCT

Elmo3 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Elmo3 siRNA (m): sc-44582 and Elmo3 shRNA Plasmid (m): sc-44582-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

Elmo3 shRNA (m) Lentiviral Particles is recommended for the inhibition of Elmo3 expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l, 528 bp). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.