

Enigma shRNA (m) Lentiviral Particles: sc-77274-V

BACKGROUND

Enigma, also known as PDLIM7 (PDZ and LIM domain 7) or LMP1 (LIM mineralization protein), is a 457 amino acid protein that localizes to both the cytoplasm and the cytoskeleton. Expressed ubiquitously with highest expression in skeletal muscle, spleen, lung and fetal liver, Enigma is thought to function as a scaffold on which protein assembly can occur. Enigma contains three LIM zinc-binding domains and one PDZ domain through which it may also act as an adaptor, linking various proteins to Actin filaments found in skeletal muscle and non-muscle tissues. Additionally, Enigma is directly involved in the two mechanisms of bone formation, namely direct bone formation (embryonic flat bones mandible and cranium) and endochondral bone formation (embryonic long bone development), and may play a role in bone fracture repair. Six isoforms of Enigma exist due to alternative splicing events.

REFERENCES

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2. Durick, K., et al. 1998. Shc and Enigma are both required for mitogenic signaling by Ret/ptc2. *Mol. Cell. Biol.* 18: 2298-2308.
3. Guy, P.M., et al. 1999. The PDZ domain of the LIM protein Enigma binds to β -Tropomyosin. *Mol. Biol. Cell* 10: 1973-1984.
4. Bach, I. 2000. The LIM domain: regulation by association. *Mech. Dev.* 91: 5-17.
5. Borrello, M.G., et al. 2002. Differential interaction of Enigma protein with the two Ret isoforms. *Biochem. Biophys. Res. Commun.* 296: 515-522.
6. Liu, Y., et al. 2002. Overexpressed LIM mineralization proteins do not require LIM domains to induce bone. *J. Bone Miner. Res.* 17: 406-414.
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CHROMOSOMAL LOCATION

Genetic locus: Pdlim7 (mouse) mapping to 13 B1.

PRODUCT

Enigma shRNA (m) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 Enigma siRNA (m): sc-77274 and Enigma shRNA Plasmid (m): sc-77274-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

Enigma (H-12): sc-398100 is recommended as a control antibody for monitoring of Enigma 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 MarkerTM 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 Enigma gene expression knockdown using RT-PCR Primer: Enigma (m)-PR: sc-77274-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.