

LIME shRNA (h) Lentiviral Particles: sc-60934-V

BACKGROUND

Lck-interacting molecule (LIME) is a 295 amino acid transmembrane adaptor protein primarily expressed in hematopoietic and lung cells. LIME has a short extracellular domain and a cytoplasmic tail containing five tyrosine-based motifs. LIME becomes tyrosine-phosphorylated after the CD4 or CD8 co-receptors cross-link. The phosphorylated LIME interacts with Lck, the Src family kinase and Csk, its negative regulator. LIME is expressed during the early and late stages of T cell activation and appears to be involved in regulation of T cell activation by co-receptors. It may be involved in activation of the ERK and JNK (both are part of the mitogen-activated protein kinase family) pathways in T cells. Bcr-mediated B cell activation may also involve LIME.

REFERENCES

1. Brdicková, N., et al. 2003. LIME: a new membrane raft-associated adaptor protein involved in CD4 and CD8 co-receptor signaling. *J. Exp. Med.* 198: 1453-1462.
2. Hur, E.M., et al. 2003. LIME, a novel transmembrane adaptor protein, associates with p56^{lck} and mediates T cell activation. *J. Exp. Med.* 198: 1463-1473.
3. Simeoni, L., et al. 2004. Adaptors and linkers in T and B cells. *Curr. Opin. Immunol.* 16: 304-313.
4. Online Mendelian Inheritance in Man, OMIM™. 2005. Johns Hopkins University, Baltimore, MD. MIM Number: 609809. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Lovatt, M., et al. 2006. Lck regulates the threshold of activation in primary T cells, while both Lck and Fyn contribute to the magnitude of the ERK response. *Mol. Cell. Biol.* 26: 8655-8665.
6. Tedoldi, S., et al. 2006. Transmembrane adaptor molecules: a new category of lymphoid-cell markers. *Blood* 107: 213-221.
7. Ahn, E., et al. 2006. LIME acts as a transmembrane adapter mediating Bcr-dependent B cell activation. *Blood* 107: 1521-1527.
8. Gregoire, C., et al. 2007. Deletion of the LIME adaptor protein minimally affects T and B cell development and function. *Eur. J. Immunol.* 37: 3259-3269.

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.

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.

CHROMOSOMAL LOCATION

Genetic locus: LIME1 (human) mapping to 20q13.33.

PRODUCT

LIME shRNA (h) 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 µl 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 LIME siRNA (h): sc-60934 and LIME shRNA Plasmid (h): sc-60934-SH as alternate gene silencing products.

APPLICATIONS

LIME shRNA (h) Lentiviral Particles is recommended for the inhibition of LIME expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

LIME (A-7): sc-365195 is recommended as a control antibody for monitoring of LIME 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 LIME gene expression knockdown using RT-PCR Primer: LIME (h)-PR: sc-60934-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.