SANTA CRUZ BIOTECHNOLOGY, INC.

LIME siRNA (m): sc-60935

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.

CHROMOSOMAL LOCATION

Genetic locus: Lime1 (mouse) mapping to 2 H4.

PRODUCT

LIME 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LIME shRNA Plasmid (m): sc-60935-SH and LIME shRNA (m) Lentiviral Particles: sc-60935-V as alternate gene silencing products.

For independent verification of LIME (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-60935A, sc-60935B and sc-60935C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

LIME siRNA (m) is recommended for the inhibition of LIME 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 µM in 66 µl. 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

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 (m)-PR: sc-60935-PR (20 µl). 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.