SANTA CRUZ BIOTECHNOLOGY, INC.

LAT siRNA (h): sc-35795

BACKGROUND

T cell receptors activate immune responses by recognizing antigen and initiating a cascade of intracellular signal transduction events, eventually culminating in cell proliferation and differentiation. Both protein tyrosine kinases and PLC γ are activated by this event. LAT, or linker for activation of T cells, is an integral membrane protein that has been shown to associate with PLC γ1, as well as GRB2 and the p85 subunit of PI 3-kinase. Binding of these signaling molecules to LAT is associated with phosphorylation of LAT by ZAP-70/Syk tyrosine kinases. LAT appears to play a role in activation of transcription mediated by AP-1 and NFAT following stimulation of the T cell receptor, suggesting that it acts as a linker protein in T cell activation. LAT protein is palmitoylated, and this modification is required for its tyrosine phosphorylation and localization to glycolipid-enriched microdomains.

REFERENCES

1. Weiss, A., et al. 1991. Signal transduction by the T cell antigen receptor. Semin. Immunol. 3: 313-324.
2. Isakov, N., et al. 1994. The role of tyrosine kinases and phosphotyrosine-containing recognition motifs in regulation of the T cell-antigen receptor-mediated signal transduction pathway. J. Leukoc. Biol. 55: 265-271.
3. Zhang, W., et al. 1998. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. Cell 92: 83-92.
4. Cantrell, D. 1998. The real LAT steps forward. Trends Cell Biol. 8: 180-182.
5. Zhang, W., et al. 1998. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity 9: 239-246.
6. Brdicka, T., et al. 1998. T cell receptor signalling results in rapid tyrosine phosphorylation of the linker protein LAT present in detergent-resistant membrane microdomains. Biochem. Biophys. Res. Commun. 248: 356-360.
7. Cho, S., et al. 2004. Structural basis for differential recognition of tyrosine-phosphorylated sites in the linker for activation of T cells (LAT) by the adaptor Gads. EMBO J. 23: 1441-1451.
8. Matsuda, S., et al. 2004. Negative feedback loop in T cell activation through MAPK-catalyzed threonine phosphorylation of LAT. EMBO J. 23: 2577-2585.
9. Bonello, G., et al. 2004. Dynamic recruitment of the adaptor protein LAT: LAT exists in two distinct intracellular pools and controls its own recruitment. J. Cell Sci. 117: 1009-1016.

CHROMOSOMAL LOCATION

Genetic locus: LAT (human) mapping to 16p11.2.

PRODUCT

LAT siRNA (h) is a target-specific 20-25 nt siRNA 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 LAT shRNA Plasmid (h): sc-35795-SH and LAT shRNA (h) Lentiviral Particles: sc-35795-V as alternate gene silencing products.

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

LAT siRNA (h) is recommended for the inhibition of LAT expression in human 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

LAT (11B.12): sc-53550 is recommended as a control antibody for monitoring of LAT 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 LAT gene expression knockdown using RT-PCR Primer: LAT (h)-PR: sc-35795-PR (20 µl, 476 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.