SANTA CRUZ BIOTECHNOLOGY, INC.

SLP-76 siRNA (h): sc-36501

BACKGROUND

The translational product of the Vav proto-oncogene is exclusively expressed in cells of hematopoietic origin and is critical for lymphocyte development and activation. However, the biochemical basis of Vav's function is unclear. Vav contains a single SH2 domain that is required for its association with the T cell receptor (TCR). Overexpression of Vav or SLP-76 in Jurkat cells leads to NFAT activation and IL-2 production. When co-expressed, Vav and SLP-76 synergize to induce a robust basal and TCR-mediated IL-2 response. Although SLP-76 does not contain a motif that would indicate it to be a member of the tyrosine, serine/threonine or lipid kinase families, it does contain several putative SH2/SH3-binding domains and has been shown to physically associate with the adapter protein GRB2 as well as PLC γ1. The discovery of SLP-76 represents an important step in elucidating the mechanism of Vav transformation and TCR-mediated NFAT activation.

REFERENCES

1. Katzav, S., et al. 1989. Vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. EMBO J. 8: 2283-2290.
2. Bustelo, X.R., et al. 1992. Tyrosine phosphorylation of the Vav proto-oncogene product in activated B cells. Science 256: 1196-1199.
3. Jackman, J.K., et al. 1995. Molecular cloning of SLP-76, a 76 kDa tyrosine phosphoprotein associated with GRB2 in T cells. J. Biol. Chem. 270: 7029-7032.
4. Hanazono, Y., et al. 1996. Proto-oncogene products Vav and c-Cbl are involved in the signal transduction through GRB2/Ash in hematopoietic cells. Acta Haematol. 95: 236-242.
5. Luger, S., et al. 1996. A functional analysis of proto-oncogene Vav's role in adult human hematopoiesis. Blood 87: 1326-1334.
6. Wu, J., et al. 1996. Vav and SLP-76 interact and functionally cooperate in IL-2 gene activation. Immunity 4: 593-602.
7. Motto, D.G., et al. 1996. Implication of the GRB2-associated phosphoprotein SLP-76 in T cell receptor-mediated interleukin-2 production. J. Exp. Med. 183: 1937-1943.

CHROMOSOMAL LOCATION

Genetic locus: LCP2 (human) mapping to 5q35.1.

PRODUCT

SLP-76 siRNA (h) 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 SLP-76 shRNA Plasmid (h): sc-36501-SH and SLP-76 shRNA (h) Lentiviral Particles: sc-36501-V as alternate gene silencing products.

For independent verification of SLP-76 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36501A, sc-36501B and sc-36501C.

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

SLP-76 siRNA (h) is recommended for the inhibition of SLP-76 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

SLP-76 (F-7): sc-13151 is recommended as a control antibody for monitoring of SLP-76 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 SLP-76 gene expression knockdown using RT-PCR Primer: SLP-76 (h)-PR: sc-36501-PR (20 µl, 524 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.