SANTA CRUZ BIOTECHNOLOGY, INC.

SKAP55-R siRNA (m): sc-40601

BACKGROUND

Fyb (Fyn binding protein) and the anchoring proteins SKAP55 (src kinase-associated phosphoprotein of 55 kDa) and SKAP55-R (SKAP55-related protein) associate with the tyrosine kinase p59Fyn. SKAP55 and SKAP55-R bind to Fyb through their SH3 domains and function as substrates for p59Fyn in resting T cells. SKAP55 contains an N-terminal pleckstrin homology domain and a C-terminal SH3 domain binding motif of adjacent arginine and lysine residues followed by tandem tyrosines (i.e. RKxxYxxY). SKAP55-R, similar in overall structure to SKAP55, contains a coiled-coil N-terminal domain. SKAP55 associates with SLAP-130, another component of the Fyn complex, which plays a role in the regulation of signaling events initiated by lymphocyte antigen receptors leading up to T cell activation. The human SKAP55 gene maps to chromosome 17q21.32 and encodes a 359 amino acid protein.

REFERENCES

1. Marie-Cardine, A., et al. 1997. Molecular cloning of SKAP55, a novel protein that associates with the protein tyrosine kinase p59fyn in human T-lymphocytes. J. Biol. Chem. 272: 16077-16080.
2. Marie-Cardine, A., et al. 1998. Molecular interaction between the Fyn-associated protein SKAP55 and the SLP-76-associated phosphoprotein SLAP-130. J. Biol. Chem. 273: 25789-25795.
3. Peterson, E.J., et al. 1998. Adaptor proteins in lymphocyte antigen-receptor signaling. Curr. Opin. Immunol. 10: 337-344.
4. Liu, J., et al. 1998. Fyb (Fyn binding protein) serves as a binding partner for lymphoid protein and Fyn kinase substrate SKAP55 and a SKAP55-related protein in T cells. Proc. Natl. Acad. Sci. USA 95: 8779-8784.
5. Marie-Cardine, A., et al. 1999. Molecular alterations of the Fyn-complex occur as late events of human T cell activation. Eur. J. Immunol. 29: 1175-1187.
6. Kang, H., et al. 2000. SH3 domain recognition of a proline-independent tyrosine-based RKxxYxxY motif in immune cell adaptor SKAP55. EMBO J. 19: 2889-2899.
7. LocusLink Report (LocusID: 8935). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: Scap2 (mouse) mapping to 6 B3.

PRODUCT

SKAP55-R 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 SKAP55-R shRNA Plasmid (m): sc-40601-SH and SKAP55-R shRNA (m) Lentiviral Particles: sc-40601-V as alternate gene silencing products.

For independent verification of SKAP55-R (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-40601A, sc-40601B and sc-40601C.

APPLICATIONS

SKAP55-R siRNA (m) is recommended for the inhibition of SKAP55-R 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

SKAP55-R (C-9): sc-398285 is recommended as a control antibody for monitoring of SKAP55-R 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 SKAP55-R gene expression knockdown using RT-PCR Primer: SKAP55-R (m)-PR: sc-40601-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.

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.