



Fyb siRNA (h): sc-40597

BACKGROUND

Fyb (Fyn binding protein) and the anchoring proteins SKAP55 (src kinase-associated phosphoprotein) 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 amino-terminal pleckstrin homology domain and a carboxy-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 Fyb gene maps to chromosome 5p13.1 and encodes a 783 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. 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.
4. Peterson, E.J., et al. 1998. Adaptor proteins in lymphocyte antigen-receptor signaling. *Curr. Opin. Immunol.* 10: 337-344.
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: 2533). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: FYB (human) mapping to 5p13.1.

PRODUCT

Fyb 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Fyb shRNA Plasmid (h): sc-40597-SH and Fyb shRNA (h) Lentiviral Particles: sc-40597-V as alternate gene silencing products.

For independent verification of Fyb (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-40597A, sc-40597B and sc-40597C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Fyb siRNA (h) is recommended for the inhibition of Fyb 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 μ M in 66 μ l. 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

Fyb (C-8): sc-515372 is recommended as a control antibody for monitoring of Fyb 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Fyb gene expression knockdown using RT-PCR Primer: Fyb (h)-PR: sc-40597-PR (20 μ l). 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.