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Sab siRNA (m): sc-153196

BACKGROUND

Sab is a Src homology 3 domain (SH3) binding protein that preferentially associates with Bruton's tyrosine kinase, Btk, over other related tyrosine kinases. Btk, together with Itk, Tec, Txk and Bmx, is a member of a family of cytoplasmic tyrosine kinases (the Btk/Tec family). Btk is a B cell specific kinase that is crucial for human and murine B cell development, and its deficiency causes human X-linked agammaglobulinemia and murine X-linked immunodeficiency. Sab serves as a negative regulator of Btk kinase activity and Sab binding to Btk reduces the phosphorylation of Btk substrates and also inhibits Btk-induced auto-phosphorylation in B cells. The SH3 domain of Sab directly binds to the SH3 domain of Btk and this interaction is essential for the regulatory activity of Sab. Sab is more broadly expressed than Btk, suggesting that Sab may target additional protein kinases that are specific to various tissues.

REFERENCES

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2. Satterthwaite, A.B., et al. 1998. Btk function in B-cell development and response. *Semin. Immunol.* 10: 309-316.
3. Yamadori, T., et al. 1999. Bruton's tyrosine kinase activity is negatively regulated by Sab, the Btk-SH3 domain-binding protein. *Proc. Natl. Acad. Sci. USA* 96: 6341-6346.
4. Kawakami, Y., et al. 1999. Functions of Bruton's tyrosine kinase in mast and B-cells. *J. Leukoc. Biol.* 65: 286-290.
5. Morrogh, L.M., et al. 1999. The SH3 domain of Bruton's tyrosine kinase displays altered ligand binding properties when auto-phosphorylated *in vitro*. *Eur. J. Immunol.* 29: 2269-2279.
6. Hashimoto, S., et al. 1999. Identification of the SH2 domain binding protein of Bruton's tyrosine kinase as BLNK—functional significance of Btk-SH2 domain in B-cell antigen receptor-coupled calcium signaling. *Blood* 94: 2357-2364.

CHROMOSOMAL LOCATION

Genetic locus: Sh3bp5 (mouse) mapping to 14 B.

PRODUCT

Sab 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Sab shRNA Plasmid (m): sc-153196-SH and Sab shRNA (m) Lentiviral Particles: sc-153196-V as alternate gene silencing products.

For independent verification of Sab (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-153196A, sc-153196B and sc-153196C.

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

Sab siRNA (m) is recommended for the inhibition of Sab 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 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

Sab (A-3): sc-390512 is recommended as a control antibody for monitoring of Sab 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 Sab gene expression knockdown using RT-PCR Primer: Sab (m)-PR: sc-153196-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.