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SPA-1 siRNA (h): sc-45418

BACKGROUND

The SPA-1 (signal-induced proliferation-associated gene-1) protein is a principal Rap1 GTPase-activating protein in the hematopoietic progenitors and peripheral T cells. The SPA-1 gene is normally expressed in fetal and adult lymphohematopoietic tissues. Various types of mitogenic stimulation increase SPA-1 mRNA expression in normal lymphocytes. SPA-1 disrupts LFA-1-ICAM1-mediated adhesive interactions and subsequent T cell-receptor triggering and IL-2 production, possibly through inhibition of RAP1. Mice that are deficient for the SPA-1 gene develop age-dependent progression of T cell immunodeficiency followed by a spectrum of late onset myeloproliferative disorders, mimicking human chronic myeloid leukemia. SPA-1 also directly binds to aquaporin-2 (AQP2) and plays a role in regulating AQP2 trafficking to the apical membrane.

REFERENCES

- Hattori, M., et al. 1995. Molecular cloning of a novel mitogen-inducible nuclear protein with a Ran GTPase-activating domain that affects cell cycle progression. *Mol. Cell. Biol.* 15: 552-560.
- Katagiri, K., et al. 2002. Rap1 functions as a key regulator of T cell and antigen-presenting cell interactions and modulates T cell responses. *Mol. Cell. Biol.* 22: 1001-1015.
- Ishida, D., et al. 2003. Antigen-driven T cell anergy and defective memory T cell response via deregulated Rap1 activation in SPA-1-deficient mice. *Proc. Natl. Acad. Sci. USA* 100: 10919-10924.
- Harazaki, M., et al. 2004. Specific recruitment of SPA-1 to the immunological synapse: involvement of Actin-bundling protein actinin. *Immunol. Lett.* 92: 221-226.
- Noda, Y., et al. 2004. Aquaporin-2 trafficking is regulated by PDZ-domain containing protein SPA-1. *FEBS Lett.* 568: 139-145.
- Kometani, K., et al. 2004. Rap1 and SPA-1 in hematologic malignancy. *Trends Mol. Med.* 10: 401-408.

CHROMOSOMAL LOCATION

Genetic locus: SIPA1 (human) mapping to 11q13.1.

PRODUCT

SPA-1 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 SPA-1 shRNA Plasmid (h): sc-45418-SH and SPA-1 shRNA (h) Lentiviral Particles: sc-45418-V as alternate gene silencing products.

For independent verification of SPA-1 (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-45418A, sc-45418B and sc-45418C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support 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 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

SPA-1 siRNA (h) is recommended for the inhibition of SPA-1 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

SPA-1 (B-7): sc-166219 is recommended as a control antibody for monitoring of SPA-1 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 SPA-1 gene expression knockdown using RT-PCR Primer: SPA-1 (h)-PR: sc-45418-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.