SANTA CRUZ BIOTECHNOLOGY, INC.

SARA siRNA (h): sc-36458

BACKGROUND

SARA (NSP, SARA, MADHIP, SMADIP, ZFYVE9, zinc finger FYVE domain containing 9) is a double zinc finger (FYVE domain) protein that influences the recruitment of Smad proteins to the TGFβ receptor and ensures appropriate subcellular localization of the activated receptor-bound complex. The FYVE domain in SARA directs localization to early endosomal compartments where it can interact with TGFβ receptors and Smads. Promyelocytic leukemia (PML) tumor suppressor physically interacts with Smad2/3 and SARA and promotes association and accumulation of SARA and TGFβ receptor in early endosome. SARA can enhance recruitment of protein phosphatase 1 catalytic subunit (PP1c) to Smad7-GADD34 complex by controlling the specific subcellular localization of PP1c. Dephosphorylation of TGFβ receptor by Smad7 is an effective mechanism for governing negative feedback in TGFβ signaling.

REFERENCES

1. Nakao, A., et al. 1997. TGFβ receptor-mediated signalling through Smad2, Smad3 and Smad4. EMBO J. 16: 5353-5362.
2. Heldin, C.H., et al. 1997. TGFβ signalling from cell membrane to nucleus through SMAD proteins. Nature 390: 465-471.
3. Derynck, R., et al. 1998. Smads: transcriptional activators of TGFβ responses. Cell 95: 737-740.
4. Tsukazaki, T., et al. 1998. SARA, a FYVE domain protein that recruits Smad2 to the TGFβ receptor. Cell 95: 779-791.
5. Wurmser, A.E., et al, 1999. Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways. J. Biol. Chem. 274: 9129-9132.

CHROMOSOMAL LOCATION

Genetic locus: ZFYVE9 (human) mapping to 1p32.3.

PRODUCT

SARA 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 SARA shRNA Plasmid (h): sc-36458-SH and SARA shRNA (h) Lentiviral Particles: sc-36458-V as alternate gene silencing products.

For independent verification of SARA (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-36458A, sc-36458B and sc-36458C.

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

SARA siRNA (h) is recommended for the inhibition of SARA 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

SARA (B-9): sc-74493 is recommended as a control antibody for monitoring of SARA 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 SARA gene expression knockdown using RT-PCR Primer: SARA (h)-PR: sc-36458-PR (20 µl, 434 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Runyan, C.E., et al. 2009. Role of SARA (SMAD anchor for receptor activation) in maintenance of epithelial cell phenotype. J. Biol. Chem. 284: 25181-25189.

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.