SANTA CRUZ BIOTECHNOLOGY, INC.

PIASx siRNA (h): sc-40849

BACKGROUND

The IL-6-type family of cytokines, which includes IL-6 and a number of similar cytokines and growth factors, plays a significant role in regulating gene activation, proliferation and differentiation. Transcription factors of the Stat (signal transducer and activator of transcription) family are involved in IL-6 family-mediated signal transduction pathways and, upon activation, undergo phosphorylation, dimerization, and translocation to the nucleus. The duration and intensity of a cell's response to cytokines can be adjusted by the effect of several regulatory mechanisms. One example involves the protein inhibitor of activated Stat family (PIAS family) of proteins, which act as negative regulators of Stats in cytokine signaling. PIAS proteins are able to coactivate steroid receptor-dependent transcription as well. PIASx transcript is alternatively spliced to yield two protein isoforms, PIASx-α and PIASx-β, which differ in their C-terminal regions. Similar to other members of the PIAS family, the predicted PIASx proteins contain a putative zinc-binding motif and a highly acidic region.

REFERENCES

1. Akira, S., et al. 1994. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77: 63-71.
2. Zhong, Z., et al. 1994. Stat3: a Stat family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 264: 95-98.
3. Heinrich, P.C., et al. 1998. Interleukin-6-type cytokine signalling through the gp130/JAK/Stat pathway. Biochem. J. 334: 297-314.
4. Liu, B., et al. 1998. Inhibition of Stat1-mediated gene activation by PIAS1. Proc. Natl. Acad. Sci. USA 95: 10626-10631.
5. Starr, R., et al. 1999. Negative regulation of the JAK/Stat pathway. Bioessays 21: 47-52.
6. Kotaja, N., et al. 2000. ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated Stat) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol. Endocrinol. 14: 1986-2000.

CHROMOSOMAL LOCATION

Genetic locus: PIAS2 (human) mapping to 18q21.1.

PRODUCT

PIASx 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 PIASx shRNA Plasmid (h): sc-40849-SH and PIASx shRNA (h) Lentiviral Particles: sc-40849-V as alternate gene silencing products.

For independent verification of PIASx (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-40849A, sc-40849B and sc-40849C.

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

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

PIASx (D-12): sc-166494 is recommended as a control antibody for monitoring of PIASx 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 PIASx gene expression knockdown using RT-PCR Primer: PIASx (h)-PR: sc-40849-PR (20 µl, 450 bp). 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.