

PIASy siRNA (h): sc-40851

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 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 signal transducer and activator of transcription (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. Human PIASy is a 510 amino acid transcriptional corepressor of the androgen receptor (AR). In addition, PIASy may regulate p53-mediated events and may direct p53 into a transactivation-independent mode of apoptosis.

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.

CHROMOSOMAL LOCATION

Genetic locus: PIAS4 (human) mapping to 19p13.3.

PRODUCT

PIASy 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 PIASy shRNA Plasmid (h): sc-40851-SH and PIASy shRNA (h) Lentiviral Particles: sc-40851-V as alternate gene silencing products.

For independent verification of PIASy (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-40851A, sc-40851B and sc-40851C.

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

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

PIASy (C-11): sc-166706 is recommended as a control antibody for monitoring of PIASy 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 PIASy gene expression knockdown using RT-PCR Primer: PIASy (h)-PR: sc-40851-PR (20 μ l, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Brayman, M.J., et al. 2007. MUC1 expression is repressed by protein inhibitor of activated signal transducer and activator of transcription- γ . *Mol. Endocrinol.* 21: 2725-2737.
2. Malewicz, M., et al. 2011. Essential role for DNA-PK-mediated phosphorylation of NR4A nuclear orphan receptors in DNA double-strand break repair. *Genes Dev.* 25: 2031-2040.

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.