SANTA CRUZ BIOTECHNOLOGY, INC.

Act1 siRNA (h): sc-29634

BACKGROUND

Members of the NFκB family of transcription factors are important in regulating the expression of various cellular and viral genes involved in immune and inflammatory responses, cell survival and stress responses. IL-1, TNFα and other related signaling pathways activate transcription factors through the activation of JNK. The NFκB signaling pathway converges with the signal-induced activation of JNK upstream of IKK. Isolated from the human embryonic kidney (HEK) 293 cell line, Act1 is an IKKγ-associated protein that activates both NFκB and JNK constitutively. Act1, also designated NFκB activator 1 or CIKS (for connection to IKK and SAPK/JNK), may function as a coordinator between two stress-induced signaling pathways.

CHROMOSOMAL LOCATION

Genetic locus: TRAF3IP2 (human) mapping to 6q21.

PRODUCT

Act1 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 Act1 shRNA Plasmid (h): sc-29634-SH and Act1 shRNA (h) Lentiviral Particles: sc-29634-V as alternate gene silencing products.

For independent verification of Act1 (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-29634A, sc-29634B and sc-29634C.

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

Act1 siRNA (h) is recommended for the inhibition of Act1 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

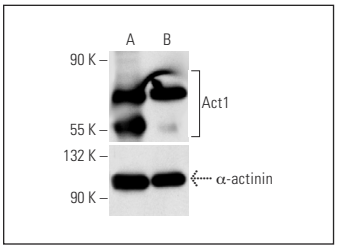
Act1 (D-11): sc-398161 is recommended as a control antibody for monitoring of Act1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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 Act1 gene expression knockdown using RT-PCR Primer: Act1 (h)-PR: sc-29634-PR (20 µl, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



Act1 siRNA (h): sc-29634. Western blot analysis of Act1 expression in non-transfected control (**A**) and Act1 siRNA transfected (**B**) HeLa cells. Blot probed with Act1 (H-300): sc-11444. α-actinin (H-2): sc-17829 used as specificity and loading control.

SELECT PRODUCT CITATIONS

1. Cho, K.A., et al. 2012. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. Int. Immunol. 24: 147-158.
2. Kim, B.K., et al. 2016. Heat shock protein 90 is involved in IL-17-mediated skin inflammation following thermal stimulation. Int. J. Mol. Med. 38: 650-658.

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.