

# Act1 siRNA (m): sc-29635

## BACKGROUND

Members of the NF $\kappa$ B 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, TNF $\alpha$  and other related signaling pathways activate transcription factors through the activation of JNK. The NF $\kappa$ B 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 IKK $\gamma$ -associated protein that activates both NF $\kappa$ B and JNK constitutively. Act1, also designated NF $\kappa$ B activator 1 or CIKS (for connection to IKK and SAPK/JNK), may function as a coordinator between two stress-induced signaling pathways.

## REFERENCES

1. Siebenlist, U., et al. 1994. Structure, regulation and function of NF $\kappa$ B. *Annu. Rev. Cell Biol.* 10: 405-455.
2. Barnes, P.J. and Karin, M. 1997. Nuclear factor- $\kappa$ B: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* 336: 1066-1071.
3. Karin, M. and Ben-Neriah, Y. 2000. Phosphorylation meets ubiquitination: the control of NF $\kappa$ B activity. *Annu. Rev. Immunol.* 18: 621-663.
4. Li, X., et al. 2000. Act1, an NF $\kappa$ B-activating protein. *Proc. Natl. Acad. Sci. USA* 97: 10489-10493.
5. Leonardi, A., et al. 2000. CIKS, a connection to I $\kappa$ B kinase and stress-activated protein kinase. *Proc. Natl. Acad. Sci. USA* 97: 10494-10499.
6. Mauro, C., et al. 2003. Role of the adaptor protein CIKS in the activation of the IKK complex. *Biochem. Biophys. Res. Commun.* 309: 84-90.

## CHROMOSOMAL LOCATION

Genetic locus: Traf3ip2 (mouse) mapping to 10 B1.

## PRODUCT

Act1 siRNA (m) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Act1 shRNA Plasmid (m): sc-29635-SH and Act1 shRNA (m) Lentiviral Particles: sc-29635-V as alternate gene silencing products.

For independent verification of Act1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29635A, sc-29635B and sc-29635C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Act1 siRNA (m) is recommended for the inhibition of Act1 expression in mouse 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  $\mu$ M in 66  $\mu$ 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

Act1 (WW-18): sc-100647 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> 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 (m)-PR: sc-29635-PR (20  $\mu$ l, 594 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Dong, Z., et al. 2013. siRNA-Act1 inhibits the function of IL-17 on lung fibroblasts via the NF- $\kappa$ B pathway. *Respiration* 86: 332-340.
2. Du, S., et al. 2020. IL-17 stimulates the expression of CCL2 in cardiac myocytes via Act1/TRAF6/p38MAPK-dependent AP-1 activation. *Scand. J. Immunol.* 91: e12840.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.