

# I $\kappa$ B- $\alpha$ siRNA (m): sc-29361

## BACKGROUND

On the basis of both functional and structural considerations, members of the I $\kappa$ B family of proteins can be divided into four groups. The first of these groups, I $\kappa$ B- $\alpha$ , includes the avian protein pp40 and the mammalian MAD-3, both of which inhibit binding of p50-p65 NF $\kappa$ B complex or Rel protein to their cognate binding sites but do not inhibit the binding of p50 homodimer to  $\kappa$ B sites, suggesting that the I $\kappa$ B- $\alpha$  family binds to the p65 subunit of p50-p65 heterocomplex through ankyrin repeats. The second member of the I $\kappa$ B family is represented by a protein designated I $\kappa$ B- $\beta$ . The third group of I $\kappa$ B proteins is represented by I $\kappa$ B- $\gamma$ , which is identical in sequence with the C-terminal domain of the p110 precursor of NF $\kappa$ B p50 and is expressed predominantly in lymphoid cells. An additional I $\kappa$ B family member, I $\kappa$ B- $\epsilon$ , has several phosphorylated forms and is primarily found complexed with Rel A and/or c-Rel.

## REFERENCES

1. Ghosh, S., et al. 1990. Activation *in vitro* to NF $\kappa$ B by phosphorylation of its inhibitor I $\kappa$ B. *Nature* 344: 678-682.
2. Kerr, L.D., et al. 1991. The Rel-associated pp40 protein prevents DNA binding of Rel and NF $\kappa$ B: relationship with I $\kappa$ B- $\beta$  and regulation by phosphorylation. *Genes Dev.* 5: 1464-1476.
3. Davis, N., et al. 1991. Rel-associated pp40: an inhibitor of the Rel family of transcription factors. *Science* 252: 1268-1271.
4. Haskill, S., et al. 1991. Characterization of an immediate-early gene induced in adherent monocytes that encodes I $\kappa$ B-like activity. *Cell* 65: 1281-1289.

## CHROMOSOMAL LOCATION

Genetic locus: Nfkb1a (mouse) mapping to 12 C1.

## PRODUCT

I $\kappa$ B- $\alpha$  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 I $\kappa$ B- $\alpha$  shRNA Plasmid (m): sc-29361-SH and I $\kappa$ B- $\alpha$  shRNA (m) Lentiviral Particles: sc-29361-V as alternate gene silencing products.

For independent verification of I $\kappa$ B- $\alpha$  (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-29361A, sc-29361B and sc-29361C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$  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

I $\kappa$ B- $\alpha$  siRNA (m) is recommended for the inhibition of I $\kappa$ B- $\alpha$  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

I $\kappa$ B- $\alpha$  (H-4): sc-1643 is recommended as a control antibody for monitoring of I $\kappa$ B- $\alpha$  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>™</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 I $\kappa$ B- $\alpha$  gene expression knockdown using RT-PCR Primer: I $\kappa$ B- $\alpha$  (m)-PR: sc-29361-PR (20  $\mu$ l, 464 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.

## SELECT PRODUCT CITATIONS

1. Takase, O., et al. 2008. Inhibition of NF $\kappa$ B-dependent Bcl-x $_L$  expression by clusterin promotes albumin-induced tubular cell apoptosis. *Kidney Int.* 73: 567-577.
2. Takase, O., et al. 2011. Eicosapentaenoic acid regulates I $\kappa$ B- $\alpha$  and prevents tubulointerstitial injury in kidney. *Eur. J. Pharmacol.* 669: 128-135.
3. Huang, R.L., et al. 2014. LPS-stimulated inflammatory environment inhibits BMP-2-induced osteoblastic differentiation through crosstalk between TLR4/MyD88/NF $\kappa$ B and BMP/Smad signaling. *Stem Cells Dev.* 23: 277-289.
4. Ye, Y., et al. 2015. Inhibition of p-I $\kappa$ B $\alpha$  ubiquitylation by autophagy-related gene 7 to regulate inflammatory responses to bacterial infection. *J. Infect. Dis.* 212: 1816-1826.

## RESEARCH USE

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