

I κ B- ϵ siRNA (h): sc-35642

BACKGROUND

On the basis of both functional and structural considerations, members of the I κ B family of proteins can be divided into four groups. The first of these groups, I κ B- α , includes the avian protein pp40 and the mammalian MAD-3, both of which inhibit binding of p50-p65 NF κ B complex or Rel protein to their cognate binding sites but do not inhibit the binding of p50 homodimer to κ B sites, suggesting that the I κ B- α family binds to the p65 subunit of p50-p65 heterocomplex through ankyrin repeats. The second member of the I κ B family is represented by a protein designated I κ B- β . The third group of I κ B proteins is represented by I κ B- γ , a protein identical in sequence with the C-terminal domain of the p110 precursor of NF κ B p50 and expressed predominantly in lymphoid cells. An additional I κ B family member has been identified as I κ B- ϵ , a protein which 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 κ B by phosphorylation of its inhibitor I κ B. *Nature* 344: 678-682.
2. Kerr, L.D., et al. 1991. The Rel-associated pp40 protein prevents DNA binding of Rel and NF κ B: relationship with I κ B- β 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 κ B-like activity. *Cell* 65: 1281-1289.
5. Inoue, J.I., et al. 1992. I κ B- γ , a 70 kd protein identical to the C-terminal half of p110 NF κ B; a new member of the I κ B family. *Cell* 68: 1109-1120.
6. Thompson, J.E., et al. 1995. I κ B- β regulates the persistent response in biphasic activation of NF κ B. *Cell* 80: 573-582.
7. Whiteside, S.T., et al. 1997. I κ B- ϵ , a novel member of the I κ B family, controls RelA and cRel NF κ B activity. *EMBO J.* 16: 1413-1426.
8. Simeonidis, S., et al. 1997. Cloning and functional characterization of mouse I κ B- ϵ . *Proc. Natl. Acad. Sci. USA* 94: 14372-14377.

CHROMOSOMAL LOCATION

Genetic locus: NFKBIE (human) mapping to 6p21.1.

PRODUCT

I κ B- ϵ 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 I κ B- ϵ shRNA Plasmid (h): sc-35642-SH and I κ B- ϵ shRNA (h) Lentiviral Particles: sc-35642-V as alternate gene silencing products.

For independent verification of I κ B- ϵ (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-35642A, sc-35642B and sc-35642C.

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

I κ B- ϵ siRNA (h) is recommended for the inhibition of I κ B- ϵ 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

I κ B- ϵ (G-4): sc-7275 is recommended as a control antibody for monitoring of I κ B- ϵ 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 I κ B- ϵ gene expression knockdown using RT-PCR Primer: I κ B- ϵ (h)-PR: sc-35642-PR (20 μ l, 509 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.