SANTA CRUZ BIOTECHNOLOGY, INC.

IκB-α siRNA (h2): sc-44265

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The Power to Question

BACKGROUND

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

CHROMOSOMAL LOCATION

Genetic locus: NFKBIA (human) mapping to 14q13.2.

PRODUCT

IκB-α siRNA (h2) 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 IκB-α shRNA Plasmid (h2): sc-44265-SH and IκB-α shRNA (h2) Lentiviral Particles: sc-44265-V as alternate gene silencing products.

For independent verification of IκB-α (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44265A, sc-44265B and sc-44265C.

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

IκB-α siRNA (h2) is recommended for the inhibition of IκB-α 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

p-IκB-α (B-9): sc-8404 is recommended as a control antibody for monitoring of IκB-α 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, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A 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 IκB-α gene expression knockdown using RT-PCR Primer: IκB-α (h2)-PR: sc-44265-PR (20 µl). 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.