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# IkB- $\alpha$ shRNA (h2) Lentiviral Particles: sc-44265-V

## BACKGROUND

On the basis of both functional and structural considerations, members of the IkB family of proteins can be divided into four groups. The first of these groups, IkB- $\alpha$ , 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 IkB- $\alpha$  family binds to the p65 subunit of p50-p65 heterocomplex through ankyrin repeats. The second member of the IkB family is represented by a protein designated IkB- $\beta$ . The third group of IkB proteins is represented by IkB- $\gamma$ , 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 IkB family member, IkB- $\epsilon$ , 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 IkB. *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 IkB- $\beta$  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 IkB-like activity. *Cell* 65: 1281-1289.
5. Inoue, J., et al. 1992. IkB- $\gamma$ , a 70 kDa protein identical to the C-terminal half of p110 NF $\kappa$ B; a new member of the IkB family. *Cell* 68: 1109-1120.
6. Thompson, J.E., et al. 1995. IkB- $\beta$  regulates the persistent response in biphasic activation of NF $\kappa$ B. *Cell* 80: 573-582.

## CHROMOSOMAL LOCATION

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

## PRODUCT

IkB- $\alpha$  shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see IkB- $\alpha$  siRNA (h2): sc-44265 and IkB- $\alpha$  shRNA Plasmid (h2): sc-44265-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

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

## APPLICATIONS

IkB- $\alpha$  shRNA (h2) Lentiviral Particles is recommended for the inhibition of IkB- $\alpha$  expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

IkB- $\alpha$  (B-3): sc-373893 is recommended as a control antibody for monitoring of IkB- $\alpha$  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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgA-HRP: sc-3791 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgA-FITC: sc-3692 (dilution range: 1:100-1:400) or goat anti-mouse IgA-TR: sc-3693 (dilution range: 1:100-1:400) with UltraCruz<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IkB- $\alpha$  gene expression knockdown using RT-PCR Primer: IkB- $\alpha$  (h2)-PR: sc-44265-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.