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# I $\kappa$ B- $\zeta$ shRNA (m) Lentiviral Particles: sc-44897-V

## BACKGROUND

I $\kappa$ B- $\zeta$  (also called MAIL-S or INAP) is a member of the I $\kappa$ B family. It shares a 30% identity with other family members and consists of six ankyrin repeats at its C-terminal. I $\kappa$ B- $\zeta$  accumulates in the nucleus and, in humans, associates with the p50 and p65 subunits of nuclear NF $\kappa$ B via its ankyrin repeats. The mouse homologue of I $\kappa$ B- $\zeta$  has only been shown to associate with the p50 subunit. I $\kappa$ B- $\zeta$  inhibits DNA binding and activity of the transcription factor NF $\kappa$ B. Distinct from other I $\kappa$ B family members, I $\kappa$ B- $\zeta$  is not degraded upon cell stimulation and activation of NF $\kappa$ B, rather evidence shows that it is up-regulated under these circumstances. This suggests that I $\kappa$ B- $\zeta$  plays a significant role in regulation of NF $\kappa$ B and that NF $\kappa$ B may regulate I $\kappa$ B- $\zeta$  in a negative feedback loop. Regulation of NF $\kappa$ B by I $\kappa$ B- $\zeta$  may differ depending on the species.

## REFERENCES

1. Yamazaki, S., et al. 2001. A novel I $\kappa$ B protein, I $\kappa$ B- $\zeta$ , induced by proinflammatory stimuli, negatively regulates NF $\kappa$ B in the nuclei. *J. Biol. Chem.* 276: 27657-27662.
2. Eto, A., et al. 2003. Essential roles for NF $\kappa$ B and a Toll/IL-1 receptor domain-specific signal(s) in the induction of I $\kappa$ B- $\zeta$ . *Biochem. Biophys. Res. Commun.* 301: 495-501.
3. Muta, T., et al. 2003. I $\kappa$ B- $\zeta$ , a new anti-inflammatory nuclear protein induced by lipopolysaccharide, is a negative regulator for NF $\kappa$ B. *J. Endotoxin. Res.* 9: 187-191.
4. Motoyama, M., et al. 2005. Positive and negative regulation of NF $\kappa$ B-mediated transcription by I $\kappa$ B- $\zeta$ , an inducible nuclear protein. *J. Biol. Chem.* 280: 7444-7451.
5. Yamazaki, S., et al. 2005. Stimulus-specific induction of a novel NF $\kappa$ B regulator, I $\kappa$ B- $\zeta$ , via Toll/Interleukin-1 receptor is mediated by mRNA stabilization. *J. Biol. Chem.* 280: 1678-1687.
6. Muta, T., et al. 2006. I $\kappa$ B- $\zeta$ : an inducible regulator of nuclear factor- $\kappa$ B. *Vitam. Horm.* 74:301-316.

## CHROMOSOMAL LOCATION

Genetic locus: Nfkbiz (mouse) mapping to 16 C1.1.

## PRODUCT

I $\kappa$ B- $\zeta$  shRNA (m) 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 I $\kappa$ B- $\zeta$  siRNA (m): sc-44897 and I $\kappa$ B- $\zeta$  shRNA Plasmid (m): sc-44897-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

I $\kappa$ B- $\zeta$  shRNA (m) Lentiviral Particles is recommended for the inhibition of I $\kappa$ B- $\zeta$  expression in mouse 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

I $\kappa$ B- $\zeta$  (H-50): sc-66935 is recommended as a control antibody for monitoring of I $\kappa$ B- $\zeta$  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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor I $\kappa$ B- $\zeta$  gene expression knockdown using RT-PCR Primer: I $\kappa$ B- $\zeta$  (m)-PR: sc-44897-PR (20  $\mu$ l, 502 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

## PROTOCOLS

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