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# NIK shRNA (h2) Lentiviral Particles: sc-44314-V

## BACKGROUND

The NF $\kappa$ B transcription factor can be activated by several cytokines including TNF and IL-1. The TNF receptor activates NF $\kappa$ B through the TRAF2 adapter protein, whereas the IL-1 receptor activates NF $\kappa$ B in a pathway involving TRAF6. Both TRAF2 and TRAF6 have been shown to interact with a Serine/Threonine kinase designated NF $\kappa$ B inducing kinase (NIK), which appears to participate in the NF $\kappa$ B signaling cascades triggered by both TNF and IL-1. NIK associates with, and is a costimulator for I $\kappa$ B kinase  $\alpha$  (IKK $\alpha$ ). IKK $\alpha$ , in turn, phosphorylates I $\kappa$ B, resulting in I $\kappa$ B degradation and NF $\kappa$ B activation. NIK has sequence similarity to several kinases that participate in MAP kinase cascades. NIK appears to be uninvolved in the TRAF2-mediated activation of JNK by TNF.

## REFERENCES

1. Rothe, M., et al. 1995. TRAF2-mediated activation of NF $\kappa$ B by TNF receptor 2 and CD40. *Science* 269: 1424-1427.
2. Hsu, H., et al. 1996. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 84: 299-308.
3. Cao, Z., et al. 1996. TRAF6 is a signal transducer for interleukin-1. *Nature* 383: 443-446.
4. Malinin, N.L., et al. 1997. MAP3K-related kinase involved in NF $\kappa$ B induction by TNF, CD95 and IL-1. *Nature* 385: 540-544.
5. Song, H.Y., et al. 1997. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor- $\kappa$ B and c-Jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc. Nat. Acad. Sci. USA* 94: 9792-9796.

## CHROMOSOMAL LOCATION

Genetic locus: MAP3K14 (human) mapping to 17q21.31.

## PRODUCT

NIK 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 NIK siRNA (h2): sc-44314 and NIK shRNA Plasmid (h2): sc-44314-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

NIK shRNA (h2) Lentiviral Particles is recommended for the inhibition of NIK 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

NIK (A-12): sc-8417 is recommended as a control antibody for monitoring of NIK 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 IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (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 NIK gene expression knockdown using RT-PCR Primer: NIK (h2)-PR: sc-44314-PR (20  $\mu$ l, 572 bp). 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.