

IKK γ siRNA (m): sc-29364

BACKGROUND

The transcription factor NF κ B is retained in the cytoplasm in an inactive form by the inhibitory protein I κ B. Activation of NF κ B requires that I κ B be phosphorylated on specific serine residues, which results in targeted degradation of I κ B. I κ B kinase α (IKK α), previously designated CHUK, interacts with I κ B α and specifically phosphorylates I κ B α on Serine 32 and 36, the sites that trigger its degradation. IKK α appears to be critical for NF κ B activation in response to pro-inflammatory cytokines. Phosphorylation of I κ B by IKK α is stimulated by the NF κ B inducing kinase (NIK), which itself is a central regulator for NF κ B activation in response to TNF and IL-1. The functional IKK complex contains three subunits, IKK α , IKK β and IKK γ (also designated NEMO), and each appear to make essential contributions to I κ B phosphorylation.

REFERENCES

1. Verma, I.M., et al. 1995. Rel/NF κ B/I κ B family: intimate tales of association and dissociation. *Genes Dev.* 9: 2723-2735.
2. Thanos, D., et al. 1995. NF κ B: a lesson in family values. *Cell* 80: 529-532.
3. Connelly, M.A. and Marcu, K.B. 1995. CHUK, a new member of the helix-loop-helix and leucine zipper families of interacting proteins, contains a serine-threonine kinase catalytic domain. *Cell. Mol. Biol. Res.* 41: 537-549.
4. Malinin, N.L., et al. 1997. MAP3K-related kinase involved in NF κ B induction by TNF, CD95 and IL-1. *Nature* 385: 540-544.
5. DiDonato, J.A., et al. 1997. A cytokine-responsive I κ B kinase that activates the transcription factor NF κ B. *Nature* 388: 548-554.

CHROMOSOMAL LOCATION

Genetic locus: Ikbkg (mouse) mapping to X A7.3.

PRODUCT

IKK γ siRNA (m) 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 IKK γ shRNA Plasmid (m): sc-29364-SH and IKK γ shRNA (m) Lentiviral Particles: sc-29364-V as alternate gene silencing products.

For independent verification of IKK γ (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29364A, sc-29364B and sc-29364C.

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

IKK γ siRNA (m) is recommended for the inhibition of IKK γ expression in mouse 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

IKK γ (F-10): sc-166398 is recommended as a control antibody for monitoring of IKK γ 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 MarkerTM 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 IKK γ gene expression knockdown using RT-PCR Primer: IKK γ (m)-PR: sc-29364-PR (20 μ l, 581 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Dong, W., et al. 2012. IKK α contributes to UVB-induced VEGF expression by regulating AP-1 transactivation. *Nucleic Acids Res.* 40: 2940-2955.

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.