SANTA CRUZ BIOTECHNOLOGY, INC.

IKAP siRNA (h): sc-40692

BACKGROUND

The transcription factor NFκB is retained in the cytoplasm in an inactive form by the inhibitory protein IκB. Activation of NFκB requires that IκB be phosphorylated on specific Serine residues, which results in the targeted degradation of IκB. IκB kinase α (IKKα), previously designated CHUK, interacts with IκB-α and specifically phosphorylates IκB-α on the sites that trigger its degradation, Serines 32 and 36. IKKα appears to be critical for NFκB activation in response to proinflammatory cytokines. Phosphorylation of the IκB by IKKα is stimulated by the NFκB inducing kinase (NIK), which itself is a central regulator for NFκB activation in response to TNF and IL-1. The functional IKK complex contains three subunits, IKKα, IKKβ and IKKγ (also designated NEMO) and each appears to make essential contributions to IκB phosphorylation. IKAP (IKK-complex-associated protein) is a protein that acts as a scaffold, interacting with NIK, IKKα and IKKβ and assembling them into an active kinase complex.

REFERENCES

1. Verma, I.M., et al. 1995. Rel/NFκB/IκB family: intimate tales of association and dissociation. Genes Dev. 9: 2723-2735.
2. Thanos, D., et al. 1995. NFκB: a lesson in family values. Cell 80: 529-532.
3. Conelly, M.A., et al.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 NFκB induction by TNF, CD95 and IL-1. Nature 385: 540-544.
5. DiDonato, J.A., et al.1997. A cytokine-responsive IκB kinase that activates the transcription factor NFκB. Nature 388: 548-554.
6. Regnier, C.H., et al. 1997. Identification and characterization of an IκB kinase. Cell 90: 373-383.
7. Scheidereit, C. 1998. Signal transduction. Docking IκB kinases. Nature 395: 225-226.
8. Cohen, L., et al. 1998. IKAP is a scaffold protein of the IκB kinase complex. Nature 395: 292-296.

CHROMOSOMAL LOCATION

Genetic locus: IKBKAP (human) mapping to 9q31.3.

PRODUCT

IKAP siRNA (h) 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 IKAP shRNA Plasmid (h): sc-40692-SH and IKAP shRNA (h) Lentiviral Particles: sc-40692-V as alternate gene silencing products.

For independent verification of IKAP (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40692A, sc-40692B and sc-40692C.

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

IKAP siRNA (h) is recommended for the inhibition of IKAP 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

IKAP (H-11): sc-376509 is recommended as a control antibody for monitoring of IKAP 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, UltraCruz® Blocking Reagent: sc-516214 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 IKAP gene expression knockdown using RT-PCR Primer: IKAP (h)-PR: sc-40692-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.