SANTA CRUZ BIOTECHNOLOGY, INC.

NKRF siRNA (m): sc-72276

BACKGROUND

NKRF (NFκB repressing factor), also known as transcription factor NRF or ITBA4 protein, is a ubiquitously expressed silencer protein. It localizes to the nucleoli but is also found in the nucleoplasm and contains one of each of the following nucleic acid binding domains: G-patch and R3H. NKRF represses the basal transcription of IFN-β, IL-8 and NOS2 by directly binding to their promoters. NKRF also binds to negative regulatory elements (NREs) and directly interacts with NFκB via its N-terminus. NKRF specifically inhibits the transcriptional activity of the NFκB proteins. In addition, NKRF contains a 5'-untranslated region (UTR) that has internal ribosome entry segment (IRES) activity. The activity of the NKRF IRES module is decreased with the down regulation of the RNA-binding protein, JKTBP1.

REFERENCES

1. Nourbakhsh, M., et al. 2000. Constitutive silencing of IFN-β promoter is mediated by NRF (NFκB-repressing factor), a nuclear inhibitor of NFκB. EMBO J. 18: 6415-6425.
2. Nourbakhsh, M., et al. 2000. NRF, a nuclear inhibitor of NFκB proteins silencing interferon-β promoter. Eur. Cytokine Netw. 11: 500-501.
3. Feng, X., et al. 2002. Identification of a negative response element in the human inducible nitric-oxide synthase (hiNOS) promoter: the role of NFκB-repressing factor (NRF) in basal repression of the hiNOS gene. Proc. Natl. Acad. Sci. USA 99: 14212-14217.
4. Niedick, I., et al. 2004. Nucleolar localization and mobility analysis of the NFκB repressing factor NRF. J. Cell Sci. 117: 3447-3458.
5. Jianfeng, D., et al. 2004. Cloning of the correct full length cDNA of NFκB-repressing factor. Mol. Cells 16: 397-401.
6. Dreikhausen, U., et al. 2005. NFκB-repressing factor inhibits elongation of human immunodeficiency virus type 1 transcription by DRB sensitivity-inducing factor. Mol. Cell. Biol. 25: 7473-7483.
7. Froese, N., et al. 2005. Innate immune responses in NFκB-repressing factor-deficient mice. Mol. Cell. Biol. 26: 293-302.
8. Reboll, M.R., et al. 2007. NRF IRES activity is mediated by RNA binding protein JKTBP1 and a 14-nt RNA element. RNA 13: 1328-1340.

CHROMOSOMAL LOCATION

Genetic locus: Nkrf (mouse) mapping to X A3.3.

PRODUCT

NKRF 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NKRF shRNA Plasmid (m): sc-72276-SH and NKRF shRNA (m) Lentiviral Particles: sc-72276-V as alternate gene silencing products.

For independent verification of NKRF (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-72276A, sc-72276B and sc-72276C.

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

NKRF siRNA (m) is recommended for the inhibition of NKRF 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 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

NKRF (E-12): sc-365568 is recommended as a control antibody for monitoring of NKRF 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 NKRF gene expression knockdown using RT-PCR Primer: NKRF (m)-PR: sc-72276-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Huang, L., et al. 2013. Down-regulation of miR-301a suppresses pro-inflammatory cytokines in Toll-like receptor-triggered macrophages. Immunology 140: 314-322.

RESEARCH USE

For research use only, not for use in diagnostic procedures.