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NFκB p50 siRNA (r): sc-156175

BACKGROUND

Proteins encoded by the v-Rel viral oncogene and its cellular homolog, c-Rel, are members of a family of transcription factors that include the two subunits of the transcription factor NFκB (p50 and p65) and the *Drosophila* maternal morphogen, dorsal. These proteins share sequence homology over a region of 300 amino acids at their NH₂-terminus, the region that contains their DNA binding and dimerization domains. The DNA binding activity of NFκB is activated and rapidly transported from the cytoplasm to the nucleus in cells exposed to mitogens or growth factors. cDNAs encoding precursors for two distinct proteins have been described. These proteins, designated p105 and p100, are highly related but map on different chromosomes. The p105 (p110) precursor contains p50 at its N-terminus and a C-terminal region that when expressed as a separate molecule, designated Pdl, binds to p50 and regulates its activity.

REFERENCES

1. Meyer, R., et al. 1991. Cloning of the DNA-binding subunit of human nuclear factor κB: the level of its mRNA is strongly regulated by phorbol ester or tumor necrosis factor α. Proc. Natl. Acad. Sci. USA 88: 966-970.
2. Schmid, R.M., et al. 1991. Cloning of an NFκB subunit which stimulates HIV transcription in synergy with p65. Nature 352: 733-736.
3. Ballard, D.W., et al. 1992. The 65 kDa subunit of human NFκB functions as a potent transcriptional activator and a target for v-Rel-mediated repression. Proc. Natl. Acad. Sci. USA 89: 1875-1879.
4. Hatada, E.N., et al. 1992. The ankyrin repeat domains of the NFκB precursor p105 and the proto-oncogene Bcl-3 act as specific inhibitors of NFκB DNA binding. Proc. Natl. Acad. Sci. USA 89: 2489-2493.

CHROMOSOMAL LOCATION

Genetic locus: Nfkb1 (rat) mapping to 2q43.

PRODUCT

NFκB p50 siRNA (r) 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 NFκB p50 shRNA Plasmid (r): sc-156175-SH and NFκB p50 shRNA (r) Lentiviral Particles: sc-156175-V as alternate gene silencing products.

For independent verification of NFκB p50 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156175A, sc-156175B and sc-156175C.

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

NFκB p50 siRNA (r) is recommended for the inhibition of NFκB p50 expression in rat 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

NFκB p50 (E-10): sc-8414 is recommended as a control antibody for monitoring of NFκB p50 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 NFκB p50 gene expression knockdown using RT-PCR Primer: NFκB p50 (r)-PR: sc-156175-PR (20 μl, 481 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Mizukoshi, T., et al. 2013. Failure in activation of the canonical NFκB pathway by human T-cell leukemia virus type 1 Tax in non-hematopoietic cell lines. Virology 443: 226-235.

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.