

NF-YA siRNA (m): sc-29948

BACKGROUND

The CCAAT-binding factor NF-Y is a heteromeric transcription factor that specifically binds to CCAAT sequences in many eukaryotic genes. NF-Y is made up of three subunits, NF-YA, NF-YB, and NF-YC. All three components are necessary for DNA binding. In each NF-Y subunit, the segment needed for formation of the NF-Y-DNA complex is conserved from yeast to human. These conserved segments are homologous to the histone-fold motif of eukaryotic histones. The DNA binding domains of the NF-YB and NF-YC subunits have been suggested to interact through a protein-protein histone-fold "handshake" motif in a manner analogous to the histone proteins, H2B and H2A, respectively.

REFERENCES

1. Baxevasis, A.D., et al. 1995. A variety of DNA-binding and multimeric proteins contain the histone fold motif. *Nucleic Acids Res.* 23: 2685-2691.
2. Sinha, S., et al. 1996. Three classes of mutations in the A subunit of the CCAAT-binding factor CBF delineate functional domains involved in the three-step assembly of the CBF-DNA complex. *Mol. Cell. Biol.* 16: 328-337.
3. Currie, R.A. 1997. Functional interaction between the DNA binding subunit trimerization domain of NF-Y and high mobility group protein HMG-I(Y). *J. Biol. Chem.* 272: 30880-30888.
4. Maity, S.N., et al. 1998. Role of the CCAAT-binding protein CBF/NF-Y in transcription. *Trends Biochem. Sci.* 23: 174-178.
5. Liang, S.G., et al. 1998. Pathway of complex formation between DNA and three subunits of CBF/NF-Y. Photocross-linking analysis of DNA-protein interaction and characterization of equilibrium steps of subunit interaction and DNA binding. *J. Biol. Chem.* 273: 31590-31598.
6. Mantovani, R. 1998. A survey of 178 NF-Y binding CCAAT boxes. *Nucleic Acids Res.* 26: 1135-1143.

CHROMOSOMAL LOCATION

Genetic locus: Nfya (mouse) mapping to 17 C.

PRODUCT

NF-YA 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 NF-YA shRNA Plasmid (m): sc-29948-SH and NF-YA shRNA (m) Lentiviral Particles: sc-29948-V as alternate gene silencing products.

For independent verification of NF-YA (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-29948A, sc-29948B and sc-29948C.

PROTOCOLS

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

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

NF-YA siRNA (m) is recommended for the inhibition of NF-YA 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

NF-YA (G-2): sc-17753 is recommended as a control antibody for monitoring of NF-YA 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 Marker™ 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 NF-YA gene expression knockdown using RT-PCR Primer: NF-YA (m)-PR: sc-29948-PR (20 μ l, 506 bp). 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.