SANTA CRUZ BIOTECHNOLOGY, INC.

NF-E2 p18 siRNA (h): sc-38103

BACKGROUND

The nuclear DNA binding protein NF-E2 regulates expression of globulin genes in developing erythroid cells through interaction with upstream AP-1-like recognition sites. More specifically, NF-E2 recognizes a site containing an intact AP-1 binding motif, preceded by a G residue two base pairs upstream. NF-E2 is an obligate heterodimer composed of NF-E2 p45 and NF-E2 p18. NF-E2 p18, also known as NF-E2U or MAFK, is an ubiquitously expressed component that is related to the v-Maf oncogene. It contains a basic-leucine zipper domain that functions in DNA binding and dimerization. In addition, NF-E2 p18 may play a role in erythroid differentiation. The major component of NF-E2 is a polypeptide, designated NF-E2 p45, that belongs to the basic region-leucine zipper family of transcription factors. This subunit of NF-E2 is specifically expressed at low level in hematopoietic progenitor cells and differentiated cells of the erythroid, megakaryocyte and mast cell lineages.

REFERENCES

1. Mignotte, V., et al. 1989. Two tissue-specific factors bind the erythroid promoter of the human porphobilinogen deaminase gene. Nucleic Acids Res. 17: 37-54.
2. Philipsen, S., et al. 1990. The β-globin dominant control region: hypersensitive site 2. EMBO J. 9: 2159-2167.
3. Ney, P.A., et al. 1990. Tandem AP-1-binding sites within the human β-globin dominant control region function as an inducible enhancer in erythroid cells. Genes Dev. 4: 993-1006.
4. Jarman, A.P., et al. 1991. Characterization of the major regulatory element upstream of the human α-globin gene cluster. Mol. Cell. Biol. 11: 4679-4689.
5. Andrews, N.C., et al. 1993. Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. Nature 362: 722-728.
6. Peters, L.L., et al. 1993. Mouse microcytic anaemia caused by a defect in the gene encoding the globin enhancer-binding protein NF-E2. Nature 362: 768-770.
7. Igarashi, K., et al. 1994. Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. Nature 367: 568-572.

CHROMOSOMAL LOCATION

Genetic locus: MAFK (human) mapping to 7p22.3.

PRODUCT

NF-E2 p18 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 NF-E2 p18 shRNA Plasmid (h): sc-38103-SH and NF-E2 p18 shRNA (h) Lentiviral Particles: sc-38103-V as alternate gene silencing products.

For independent verification of NF-E2 p18 (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-38103A, sc-38103B and sc-38103C.

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

NF-E2 p18 siRNA (h) is recommended for the inhibition of NF-E2 p18 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

NF-E2 p18 (132I2G): sc-517604 is recommended as a control antibody for monitoring of NF-E2 p18 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 NF-E2 p18 gene expression knockdown using RT-PCR Primer: NF-E2 p18 (h)-PR: sc-38103-PR (20 µl, 504 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.

PROTOCOLS

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