

ATF-2 siRNA (r): sc-156017

BACKGROUND

Eukaryotic gene transcription is regulated by sequence-specific transcription factors which bind modular *cis*-acting promoter and enhancer elements. The ATF/CREB transcription factor family binds the palindromic cAMP response element (CRE) octanucleotide TGACGTC A. The ATF/CREB family includes CREB-1, CREB-2 (also designated ATF-4), ATF-1, ATF-2 and ATF-3. This family of proteins contain highly divergent N-terminal domains, but share a C-terminal leucine zipper for dimerization and DNA binding. ATF-2 forms homodimers and heterodimers with c-Jun to initiate CRE-dependent transcription. Phosphorylation of ATF-2 at Thr 69 and Thr 71 by stress-activated kinases is necessary for transcriptional activation. Myc also induces phosphorylation of ATF-2 at Thr 69 and Thr 71 to prolong the half-life of ATF-2. ATF-2 also functions as a histone acetyltransferase (HAT) by specifically acetylating histones H2B and H4 *in vitro*.

REFERENCES

1. Montminy, M.R., et al. 1986. Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. *Proc. Natl. Acad. Sci. USA* 83: 6682-6686.
2. Lin, Y.S. and Green, M.R. 1988. Interaction of a common cellular transcription factor, ATF, with regulatory elements in both E1A- and cyclic AMP-inducible promoters. *Proc. Natl. Acad. Sci. USA* 85: 3396-3400.
3. Hai, T., et al. 1989. Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes Dev.* 8: 2083-2090.
4. Diep, A., et al. 1991. Assignment of the gene for cyclic AMP-response element binding protein-2 (CREB2) to human chromosome 2q24.1-q32. *Genomics* 11: 1161-1163.

CHROMOSOMAL LOCATION

Genetic locus: *Atf2* (rat) mapping to 3q23.

PRODUCT

ATF-2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ATF-2 shRNA Plasmid (r): sc-156017-SH and ATF-2 shRNA (r) Lentiviral Particles: sc-156017-V as alternate gene silencing products.

For independent verification of ATF-2 (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-156017A, sc-156017B and sc-156017C.

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

ATF-2 siRNA (r) is recommended for the inhibition of ATF-2 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 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

p-ATF-2 (F-1): sc-8398 is recommended as a control antibody for monitoring of ATF-2 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, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A 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 ATF-2 gene expression knockdown using RT-PCR Primer: ATF-2 (r)-PR: sc-156017-PR (20 μ l, 427 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhu, W., et al. 2010. Thrombin induces endothelial arginase through AP-1 activation. *Am. J. Physiol., Cell Physiol.* 298: C952-C960.
2. Salinas-Abarca, A.B., et al. 2018. ATF2, but not ATF-3, participates in the maintenance of nerve injury-induced tactile allodynia and thermal hyperalgesia. *Mol. Pain* 14: 1744806918787427.

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.