



ATF-4 shRNA (m) Lentiviral Particles: sc-35113-V

BACKGROUND

Eukaryotic gene transcription is regulated by sequence-specific transcription factors which bind modular *cis*-acting promoter and enhancer elements. The cAMP response element (CRE), one of the best studied of such elements, consists of the palindromic octanucleotide TGACGTCA. Several CRE binding proteins have been identified within the ATF/CREB family, the best characterized of which include CREB-1, CREB-2 (also designated ATF-4), ATF-1, ATF-2 and ATF-3. These proteins share highly related COOH terminal leucine zipper dimerization and basic DNA binding domains but are highly divergent in their amino terminal domains. Although each of the ATF/CREB proteins appear capable of binding CRE in its homodimeric form, certain of these also bind as heterodimers, both within the ATF/CREB family and even with members of the AP-1 transcription factor family.

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. et al. 1988. Interaction of a common cellular transcription factor, ATF, with regulatory elements in both *Ela*- and cyclic AMP-inducible promoters. *Proc. Natl. Acad. Sci. USA* 85: 3396-4000.
3. Hoeffler, J.P., et al. 1988. Cyclic AMP-responsive DNA-binding protein: structure based on a cloned placental cDNA. *Science* 242: 1430-1433.
4. 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.
5. Maekawa, T., et al. 1989. Leucine zipper structure of the protein CREB-1 binding to the cyclic AMP response element in brain. *EMBO J.* 8: 2023-2028.

CHROMOSOMAL LOCATION

Genetic locus: *Atf4* (mouse) mapping to 15 E1.

PRODUCT

ATF-4 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ATF-4 siRNA (m): sc-35113 and ATF-4 shRNA Plasmid (m): sc-35113-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

ATF-4 shRNA (m) Lentiviral Particles is recommended for the inhibition of ATF-4 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ATF-4 (B-3): sc-390063 is recommended as a control antibody for monitoring of ATF-4 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 ATF-4 gene expression knockdown using RT-PCR Primer: ATF-4 (m)-PR: sc-35113-PR (20 μ l, 532 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, B., et al. 2017. RAGE deficiency alleviates aortic valve calcification in ApoE^{-/-} mice via the inhibition of endoplasmic reticulum stress. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863: 781-792.
2. Wang, W., et al. 2018. Down-regulation of miR-193a-3p promotes osteoblast differentiation through up-regulation of LGR4/ATF4 signaling. *Biochem. Biophys. Res. Commun.* 503: 2186-2193.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.