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Egr-1 shRNA (h2) Lentiviral Particles: sc-44203-V

BACKGROUND

Egr-1, Egr-2, Egr-3 and Egr-4 are nuclear transcription factors belonging to the Egr C₂H₂-type zinc-finger protein family and containing three C₂H₂-type zinc fingers. As immediate early proteins, Egr transcription factors are rapidly induced by diverse extracellular stimuli. They are subject to tight differential control through diverse mechanisms at several levels of regulation: transcriptional; translational and posttranslational (including glycosylation, phosphorylation and redox) mechanisms; and protein-protein interaction. Egr-1 binds to the DNA sequence 5'-CGCCCCGC-3' (Egr-site), thereby activating transcription of target genes whose products are required for mitogenesis and differentiation. Egr-2 binds specific DNA sites located in the promoter region of HoxA4. Egr-2 defects cause congenital hypo-myelination neuropathy (also designated Charcot-Marie-Tooth disease) and Dejerine-Sottas neuropathy (also designated hereditary motor and sensory neuropathy III). Egr-3 is involved in muscle spindle development and is expressed in T cells 20 minutes following activation. Egr-4 binds to the Egr consensus motif GCGTGGGCG, functions as a transcriptional repressor, and displays autoregulatory activities, down-regulating its own gene promoter in a dose dependent manner.

REFERENCES

1. Beckmann, A.M., et al. 1997. Egr transcription factors in the nervous system. *Neurochem. Int.* 31: 477-510.
2. Zipfel, P.F., et al. 1997. The human zinc finger protein EGR-4 acts as autoregulatory transcriptional repressor. *Biochim. Biophys. Acta* 1354: 134-144.
3. Chen, C.C., et al. 2004. Egr-1 is activated by 17 β -estradiol in MCF-7 cells by mitogen-activated protein kinase-dependent phosphorylation of ELK-1. *J. Cell. Biochem.* 93: 1063-1074.

CHROMOSOMAL LOCATION

Genetic locus: EGR1 (human) mapping to 5q31.2.

PRODUCT

Egr-1 shRNA (h2) 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 x 10⁶ 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 Egr-1 siRNA (h2): sc-44203 and Egr-1 shRNA Plasmid (h2): sc-44203-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

Egr-1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Egr-1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ 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

Egr-1 (C-19): sc-189 is recommended as a control antibody for monitoring of 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Egr-1 gene expression knockdown using RT-PCR Primer: Egr-1 (h2)-PR: sc-44203-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.

PROTOCOLS

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