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EBF3 shRNA (h) Lentiviral Particles: sc-43743-V

BACKGROUND

B lymphocyte maturation is an intricate process that requires a distinct set of transcription factors with respect to the stage of cell differentiation and cell lineage. Among the transcriptional regulators involved in the early stages of B cell development, early B cell factor 3 (EBF3) targets promoter elements for B lymphoid kinase (Btk) and genes encoding portions of the early stage B cell receptors (BCR), which are necessary for initiation of Ig light chain gene recombination and Src kinase (Bcr) signaling. EBF3 is a nuclear transcription factor that is highly expressed in brain and exists as either a homodimer or a heterodimer with related family members. Specifically, EBF3 exhibits transcriptional activation activity on target genes by interacting with the core DNA sequence, CCCNNGGG, through a DNA-recognition domain containing a zinc-coordination motif. Two isoforms of EBF3 exist due to alternative splicing events.

REFERENCES

1. Wang, M.M., et al. 1993. Molecular cloning of the olfactory neuronal transcription factor OLF1 by genetic selection in yeast. *Nature* 364: 121-126.
2. Lin, H., et al. 1995. Failure of B cell differentiation in mice lacking the transcription factor EBF. *Nature* 376: 263-267.
3. Hagman, J., et al. 1995. EBF contains a novel zinc-coordination motif and multiple dimerization and transcriptional activation domains. *EMBO J.* 14: 2907-2916.
4. Sigvardsson, M., et al. 1997. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. *Immunity* 7: 25-36.
5. Akerblad, P., et al. 1999. The B29 (immunoglobulin β -chain) gene is a genetic target for early B cell factor. *Mol. Cell. Biol.* 19: 392-401.

CHROMOSOMAL LOCATION

Genetic locus: EBF3 (human) mapping to 10q26.3.

PRODUCT

EBF3 shRNA (h) 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 EBF3 siRNA (h): sc-43743 and EBF3 shRNA Plasmid (h): sc-43743-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

EBF3 shRNA (h) Lentiviral Particles is recommended for the inhibition of EBF3 expression in human 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

EBF3 (N7Q): sc-81999 is recommended as a control antibody for monitoring of EBF3 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EBF3 gene expression knockdown using RT-PCR Primer: EBF3 (h)-PR: sc-43743-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.