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# PBEF shRNA (h) Lentiviral Particles: sc-45843-V

## BACKGROUND

Pre-B cell-enhancing factor (PBEF), also designated nicotinamide phosphoribosyltransferase (Nampt) or visfatin, belongs to the NAPRTase family of proteins. PBEF may be involved in enhancing the effect of IL-7 and SCF on the formation of early B-lineage precursor colonies. It is involved in the catalysis of nicotinamide with 5-phosphoribosyl-1-pyrophosphate, yielding nicotinamide mononucleotide, which is important in NAD biosynthesis. This is a rate limiting step in the NAD biosynthesis pathway. Highly enriched in the visceral fat of both human and mouse, PBEF expression levels in plasma increase during the development of obesity. PBEF is a cytoplasmic protein expressed primarily in bone marrow, muscle and liver tissue, but it can also be detected in placenta, lung, kidney and heart tissue.

## REFERENCES

- Samal, B., et al. 1994. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol. Cell. Biol.* 14: 1431-1437.
- Ognjanovic, S., et al. 2001. Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. *J. Mol. Endocrinol.* 26: 107-117.
- Martin, P.R., et al. 2001. Identification of a plasmid-encoded gene from *Haemophilus ducreyi* which confers NAD independence. *J. Bacteriol.* 183: 1168-1174.
- Ognjanovic, S., et al. 2002. Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. *Am. J. Obstet. Gynecol.* 187: 1051-1058.
- Jia, S.H., et al. 2004. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J. Clin. Invest.* 113: 1318-1327.
- Revollo, J.R., et al. 2004. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. *J. Biol. Chem.* 279: 50754-50763.

## CHROMOSOMAL LOCATION

Genetic locus: NAMPT (human) mapping to 7q22.3.

## PRODUCT

PBEF 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 PBEF siRNA (h): sc-45843 and PBEF shRNA Plasmid (h): sc-45843-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

PBEF shRNA (h) Lentiviral Particles is recommended for the inhibition of PBEF expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

PBEF (E-3): sc-393444 is recommended as a control antibody for monitoring of PBEF 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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PBEF gene expression knockdown using RT-PCR Primer: PBEF (h)-PR: sc-45843-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  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.

## 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.

## PROTOCOLS

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