

PBEF siRNA (m): sc-45844

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 mice, 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

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- 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.
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- 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.
- Ye, S.Q., et al. 2005. Pre-B-cell-colony-enhancing factor is critically involved in Thrombin-induced lung endothelial cell barrier dysregulation. *Microvasc. Res.* 70: 142-151.

CHROMOSOMAL LOCATION

Genetic locus: Nampt (mouse) mapping to 12 A3.

PRODUCT

PBEF siRNA (m) 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 PBEF shRNA Plasmid (m): sc-45844-SH and PBEF shRNA (m) Lentiviral Particles: sc-45844-V as alternate gene silencing products.

For independent verification of PBEF (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45844A, sc-45844B and sc-45844C.

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

PBEF shRNA (m) Lentiviral Particles is recommended for the inhibition of PBEF expression in mouse 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

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 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 PBEF gene expression knockdown using RT-PCR Primer: PBEF (m)-PR: sc-45844-PR (20 μ l, 512 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.