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BAF shRNA (m) Lentiviral Particles: sc-44804-V

BACKGROUND

Barrier-to-autointegration factor (BAF) binds non-specifically to double stranded DNA, possibly to play a role in tissue- or cell type-specific gene expression by interacting with different homeodomain transcription factors. BAF compresses chromatin structure and interacts with the LEM domain of nuclear proteins to play a crucial role in membrane recruitment and chromatin decondensation during nuclear assembly. Additionally, retroviruses like HIV-1 incorporate BAF from host cells into preintegration complexes (PICs) to prevent autointegration of retroviral DNA and thereby promote integration of retroviral DNA into the host chromosome.

REFERENCES

1. Cai, M., et al. 1998. Solution structure of the cellular factor BAF responsible for protecting retroviral DNA from autointegration. *Nat. Struct. Biol.* 5: 903-909.
2. Haraguchi, T., et al. 2001. BAF is required for emerin assembly into the reforming nuclear envelope. *J. Cell Sci.* 114: 4575-4585.
3. Lee, K.K., et al. 2001. Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF. *J. Cell Sci.* 114: 4567-4573.
4. Wang, X., et al. 2002. Barrier to autointegration factor interacts with the cone-rod homeobox and represses its transactivation function. *J. Biol. Chem.* 277: 43288-43300.
5. Segura-Totten, M., et al. 2002. Barrier-to-autointegration factor: major roles in chromatin decondensation and nuclear assembly. *J. Cell Biol.* 158: 475-485.
6. Mansharamani, M., et al. 2003. Barrier-to-autointegration factor BAF binds p55 Gag and matrix and is a host component of human immunodeficiency virus type 1 virions. *J. Virol.* 77: 13084-13092.

CHROMOSOMAL LOCATION

Genetic locus: Banf1 (mouse) mapping to 19 A.

PRODUCT

BAF 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 BAF siRNA (m): sc-44804 and BAF shRNA Plasmid (m): sc-44804-SH as alternate gene silencing products.

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.

APPLICATIONS

BAF shRNA (m) Lentiviral Particles is recommended for the inhibition of barrier-to-autointegration factor 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

BAF (FL-89): sc-33787 is recommended as a control antibody for monitoring of BAF 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 barrier-to-autointegration factor gene expression knockdown using RT-PCR Primer: BAF (m)-PR: sc-44804-PR (20 μ l, 449 bp). 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.