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Cabin-1 shRNA (h) Lentiviral Particles: sc-43652-V

BACKGROUND

Calcineurin binding protein (Cabin-1) and the corresponding rat homolog, designated Cain, are widely expressed nuclear phosphoproteins that regulate the serine/threonine phosphatase activity of calcineurin and influence T cell signaling and apoptosis. Calcineurin is required for the transcriptional activation of cytokines and the activation of various transcription factors, including NFAT, NF κ B and AP-1, involved in T cell receptor (TCR)-mediated signaling. The regulation of calcineurin depends on the changes in intracellular calcium concentrations and the activity of protein kinase C. TCR activation results in PKC inducing the hyperphosphorylation of Cabin-1, which facilitates the high affinity binding of Cabin-1 to calcineurin. This complex formation, in turn, inhibits calcineurin activity and attenuates TCR-mediated signaling. Cabin-1 also associates directly with MEF-2 proteins, a family of transcription factors that regulate apoptosis signaling in T cells. This association between Cabin-1 and MEF-2 leads to the inhibition of MEF-2-mediated gene transcription and the inhibition of apoptosis.

REFERENCES

- Shenolikar, S. 1994. Protein serine/threonine phosphatases—new avenues for cell regulation. *Annu. Rev. Cell Biol.* 10: 55-86.
- Black, B.L., et al. 1998. Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF-2) proteins. *Annu. Rev. Cell. Dev. Biol.* 14: 167-196.
- Sun, L., et al. 1998. Cabin-1, a negative regulator for calcineurin signaling in T lymphocytes. *Immunity* 8: 703-711.
- Lai, M.M., et al. 1998. Cain, a novel physiologic protein inhibitor of calcineurin. *J. Biol. Chem.* 273: 18325-18331.

CHROMOSOMAL LOCATION

Genetic locus: CABIN1 (human) mapping to 22q11.23.

PRODUCT

Cabin-1 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 Cabin-1 siRNA (h): sc-43652 and Cabin-1 shRNA Plasmid (h): sc-43652-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

Cabin-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Cabin-1 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

Cabin-1 (K-14): sc-12519 is recommended as a control antibody for monitoring of Cabin-1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Cabin-1 gene expression knockdown using RT-PCR Primer: Cabin-1 (h)-PR: sc-43652-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.