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SANTA CRUZ BIOTECHNOLOGY, INC.

CIB shRNA (h) Lentiviral Particles: sc-43271-V

BACKGROUND

Platelets regulate the function of Integrin α2b/β3 (GPIIb/IIIa), the platelet Fibrinogen receptor, which is involved in the binding of proteins to integrin cytoplasmic domains. A novel protein, CIB, for calcium- and integrin-binding protein (also designated as Kip for kinase interacting protein, SIP2-28 and DNA-PK_{cs} interacting protein), binds specifically at the cytoplasmic domain of α2b by a number of positively charged residues in its binding site. Binding of CIB to the α2b is affected by fluctuations in the intracellular calcium concentration. In aggregated platelets, endogenous CIB and α2b/β3 translocate to the Triton X-100-insoluble cytoskeleton, demonstrating that the cellular localization of CIB is regulated. CIB also binds to DNA-PK_{cs}, which is a nuclear protein serine/threonine kinase that plays a role in the DNA repair and recombination process of lymphoid development. Fnk also binds to the CIB, suggesting that CIB may be a regulatory subunit of polo-like kinases. CIB shows significant homology to calcineurin B and calmodulin, and its mRNA levels are ubiquitously expressed in various human tissues.

REFERENCES

1. Naik, U.P., et al. 1997. Identification of a novel calcium-binding protein that interacts with the Integrin α2b cytoplasmic domain. J. Biol. Chem. 272: 4651-4654.
2. Wu, X. and Lieber, M.R. 1997. Interaction between DNA-dependent protein kinase and a novel protein, Kip. Mutat. Res. 385: 13-20.
3. Shock, D.D., et al. 1999. Calcium-dependent properties of CIB binding to the Integrin α2b cytoplasmic domain and translocation to the platelet cytoskeleton. Biochem. J. 342: 729-735.
4. Seki, N., et al. 1999. Structure, expression profile and chromosomal location of an isolog of DNA-PK_{cs} interacting protein (Kip) gene. Biochim. Biophys. Acta 1444: 143-147.
5. Hwang, P.M. and Vogel, H.J. 2000. Structures of the platelet calcium- and integrin-binding protein and the Integrin α2b cytoplasmic domain suggest a mechanism for calcium-regulated recognition; homology modeling and NMR studies. J. Mol. Recognit. 13: 83-92.

CHROMOSOMAL LOCATION

Genetic locus: CIB1 (human) mapping to 15q26.1.

PRODUCT

CIB 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 µl frozen stock containing 1.0 x 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 CIB siRNA (h): sc-43271 and CIB shRNA Plasmid (h): sc-43271-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.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 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

CIB (E-9): sc-271041 is recommended as a control antibody for monitoring of CIB 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 CIB gene expression knockdown using RT-PCR Primer: CIB (h)-PR: sc-43271-PR (20 µl, 419 bp). 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.

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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