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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



CIN85 shRNA (m) Lentiviral Particles: sc-45333-V

BACKGROUND

CD2AP (CMS) and CIN85 (Ruk) belong to a family of ubiquitously expressed adaptor molecules containing three SH3 domains, a proline-rich region and a coiled-coil domain. By binding to numerous proteins, CD2AP and CIN85 assemble multimeric complexes implicated in cell-specific signals controlling T cell activation, kidney glomeruli function or apoptosis in neuronal cells. CIN85/CD2AP also associate with accessory endocytic proteins, components of the Actin cytoskeleton and other adaptor proteins involved in receptor tyrosine kinase signaling. These interactions enable CIN85/CD2AP to function within a network of signaling pathways that coordinate critical steps involved in downregulation and degradation of receptor tyrosine kinases.

REFERENCES

1. Take, H., et al. 2000. Cloning and characterization of a novel adaptor protein, CIN85, that interacts with c-Cbl. *Biochem. Biophys. Res. Commun.* 268: 321-328.
2. Watanabe, S., et al. 2000. Characterization of the CIN85 adaptor protein and identification of components involved in CIN85 complexes. *Biochem. Biophys. Res. Commun.* 278: 167-174.
3. Szymkiewicz, I., et al. 2002. CIN85 participates in Cbl-b-mediated downregulation of receptor tyrosine kinases. *J. Biol. Chem.* 277: 39666-39672.
4. Haglund, K., et al. 2002. Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. *Proc. Natl. Acad. Sci. USA* 99: 12191-12196.
5. Dikic, I. 2002. CIN85/CMS family of adaptor molecules. *FEBS Lett.* 529: 110-115.

CHROMOSOMAL LOCATION

Genetic locus: Sh3kbp1 (mouse) mapping to X F4.

PRODUCT

CIN85 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 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CIN85 siRNA (m): sc-45333 and CIN85 shRNA Plasmid (m): sc-45333-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

CIN85 shRNA (m) Lentiviral Particles is recommended for the inhibition of CIN85 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

CIN85 (E-1): sc-377342 is recommended as a control antibody for monitoring of CIN85 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 CIN85 gene expression knockdown using RT-PCR Primer: CIN85 (m)-PR: sc-45333-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.