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CIN85 siRNA (m): sc-45333

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 down-regulation 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.
6. Schmidt, M.H., et al. 2003. SETA/CIN85/Ruk and its binding partner AIP1 associate with diverse cytoskeletal elements, including FAKs and modulate cell adhesion. *J. Cell Sci.* 116: 2845-2855.
7. Kowanetz, K., et al. 2004. CIN85 associates with multiple effectors controlling intracellular trafficking of epidermal growth factor receptors. *Mol. Biol. Cell* 15: 3155-3166.

CHROMOSOMAL LOCATION

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

PRODUCT

CIN85 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 CIN85 shRNA Plasmid (m): sc-45333-SH and CIN85 shRNA (m) Lentiviral Particles: sc-45333-V as alternate gene silencing products.

For independent verification of CIN85 (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-45333A, sc-45333B and sc-45333C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

CIN85 siRNA (m) is recommended for the inhibition of CIN85 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

CIN85 (A-7): sc-166862 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 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 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.