

CAP siRNA (m): sc-40340

BACKGROUND

c-Cbl associated protein (CAP), also designated ponsin and SH3P12, interacts with c-Cbl and facilitates the tyrosine phosphorylation of c-Cbl in response to Insulin. CAP contains three adjacent Src homology-3 (SH3) domains in the carboxy terminus. It interacts with the focal adhesion kinase p125FAK and co-localizes with Actin stress fibers. CAP is expressed in 3T3-L1 adipocytes, but not in 3T3-L1 or NIH/3T3 fibroblasts. Expression of the CAP gene is stimulated by thiazolidinediones (TZDs) through activation of PPAR γ . In addition to its interaction with c-Cbl, CAP interacts with Sos through the same SH3 domain, and may facilitate protein-protein associations involved in cell structural changes.

REFERENCES

- Ribon, V., et al. 1998. A novel, multifunctional c-Cbl binding protein in Insulin receptor signaling in 2T3-L1 adipocytes. *Mol. Cell. Biol.* 18: 872-879.
- Ribon, V., et al. 1998. A role for CAP, a novel, multifunctional Src homology 3 domain-containing protein in formation of actin stress fibers and focal adhesions. *J. Biol. Chem.* 273: 4073-4080.
- Ribon, V., et al. 1998. Thiazolidinediones and Insulin resistance: peroxisome proliferator-activated receptor γ activation stimulates expression of the CAP gene. *Proc. Natl. Acad. Sci. USA* 95: 14751-14756.
- Kurakin, A., et al. 1998. Molecular recognition properties of the C-terminal Sh3 domain of the Cbl associated protein, Cap. *J. Pept. Res.* 52: 331-337.
- Baumann, C.A., et al. 2000. Cloning and characterization of a functional peroxisome proliferator activator receptor- γ -responsive element in the promoter of the CAP gene. *J. Biol. Chem.* 275: 9131-9135.

CHROMOSOMAL LOCATION

Genetic locus: Sorbs1 (mouse) mapping to 19 C3.

PRODUCT

CAP 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 CAP shRNA Plasmid (m): sc-40340-SH and CAP shRNA (m) Lentiviral Particles: sc-40340-V as alternate gene silencing products.

For independent verification of CAP (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-40340A, sc-40340B and sc-40340C.

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

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

CAP (G-3): sc-166903 is recommended as a control antibody for monitoring of CAP 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 CAP gene expression knockdown using RT-PCR Primer: CAP (m)-PR: sc-40340-PR (20 μ l, 496 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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