

p130 Cas siRNA (r): sc-155989

BACKGROUND

p130 represents one of several known substrates for v-Crk encoded p47. p130 Cas (for Crk-associated substrate) exhibits a high level of tyrosine phosphorylation and is tightly associated with v-Crk, suggesting a role in v-Crk-mediated cell signaling. The molecular cloning of p130 Cas has shown it to represent a novel SH3 containing signaling molecule with a cluster of multiple putative SH2-binding motifs for v-Crk. By immunoprecipitation analysis, p130 Cas has been shown to be highly phosphorylated at tyrosine residues subsequent to either v-Src p60 or v-Crk-mediated transformation and to form stable complexes with both of these transforming proteins. p130 Cas behaves as an extremely potent substrate for protein tyrosine kinases and has been reported to relocate from the cytoplasm to cell membrane upon tyrosine phosphorylation. One proposed model is that the SH2 domain of v-Crk functions to activate c-Src kinase, which in turn phosphorylates p130 Cas.

REFERENCES

1. Matsuda, M., et al. 1990. Binding of transforming protein, p47gag-Crk, to a broad range of phosphotyrosine-containing proteins. *Science* 248: 1537-1539.
2. Kanner, S.B., et al. 1990. Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc. Natl. Acad. Sci. USA* 87: 3328-3332.
3. Kanner, S.B., et al. 1991. The SH2 and SH3 domains of pp60Src direct stable association with tyrosine phosphorylated proteins p130 and p110. *EMBO J.* 10: 1689-1698.
4. Matsuda, M., et al. 1991. Identification of the v-Crk oncogene product sufficient for association with phosphotyrosine-containing proteins. *Mol. Cell. Biol.* 11: 1607-1613.
5. Birge, R.B., et al. 1992. Tyrosine-phosphorylated epidermal growth factor receptor and cellular p130 provide high affinity binding substrates to analyze Crk-phosphotyrosine-dependent interactions *in vitro*. *J. Biol. Chem.* 267: 10588-10595.
6. Matsuda, M., et al. 1992. Two species of human Crk cDNA encode proteins with distinct biological activities. *Mol. Cell. Biol.* 12: 3482-3489.

CHROMOSOMAL LOCATION

Genetic locus: Bcar1 (rat) mapping to 19q12.

PRODUCT

p130 Cas siRNA (r) 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 p130 Cas shRNA Plasmid (r): sc-155989-SH and p130 Cas shRNA (r) Lentiviral Particles: sc-155989-V as alternate gene silencing products.

For independent verification of p130 Cas (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-155989A, sc-155989B and sc-155989C.

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

p130 Cas siRNA (r) is recommended for the inhibition of p130 Cas expression in rat 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

p130 Cas (35B.1A4): sc-20029 is recommended as a control antibody for monitoring of p130 Cas 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 p130 Cas gene expression knockdown using RT-PCR Primer: p130 Cas (r)-PR: sc-155989-PR (20 μ l). 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.