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# p130 Cas shRNA (h2) Lentiviral Particles: sc-44317-V

## 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.

## CHROMOSOMAL LOCATION

Genetic locus: BCAR1 (human) mapping to 16q23.1.

## PRODUCT

p130 Cas shRNA (h2) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 p130 Cas siRNA (h2): sc-44317 and p130 Cas shRNA Plasmid (h2): sc-44317-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

p130 Cas shRNA (h2) Lentiviral Particles is recommended for the inhibition of p130 Cas expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

p130 Cas (C-4): sc-365200 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 (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 p130 Cas gene expression knockdown using RT-PCR Primer: p130 Cas (h2)-PR: sc-44317-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  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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