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CHIP shRNA (h) Lentiviral Particles: sc-43555-V

BACKGROUND

CHIP (carboxy terminus of HSP 70-interacting protein), also designated STIP1 homology and U-box containing protein 1, HSPABP2, NY-CO-7, SDCCAG7 and STUB1, is a cytoplasmic E3 ubiquitin ligase that influences protein ubiquitylation. CHIP interacts with Smad1/Smad4 and blocks BMP signaling through the ubiquitin-mediated degradation of Smad proteins. It controls both association of HSP 70/HSP 90 chaperones with ErbB-2 and downregulation of ErbB-2 induced by inhibitors of HSP 90. A 1.3 kb transcript is most abundant in striated muscle (heart and skeletal muscle), with lower expression in pancreas and brain.

REFERENCES

1. Ballinger, C.A., et al. 1999. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol. Cell. Biol.* 19: 4535-4545.
2. Jiang, J., et al. 2001. CHIP is a U-box-dependent E3 ubiquitin ligase: identification of HSC 70 as a target for ubiquitylation. *J. Biol. Chem.* 276: 42938-42944.
3. Xu, W., et al. 2002. Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB-2/Neu. *Proc. Natl. Acad. Sci. USA* 99: 12847-12852.
4. Imai, Y., et al. 2002. CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. *Mol. Cell* 10: 55-67.
5. Jiang, J., et al. 2003. Chaperone-dependent regulation of endothelial nitric-oxide synthase intracellular trafficking by the co-chaperone/ubiquitin ligase CHIP. *J. Biol. Chem.* 278: 49332-49341.
6. Schipper, R.G., et al. 2004. Intracellular localization of ornithine decarboxylase and its regulatory protein, antizyme-1. *J. Histochem. Cytochem.* 52: 1259-1266.

CHROMOSOMAL LOCATION

Genetic locus: STUB1 (human) mapping to 16p13.3.

PRODUCT

CHIP shRNA (h) 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 CHIP siRNA (h): sc-43555 and CHIP shRNA Plasmid (h): sc-43555-SH as alternate gene silencing products.

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.

APPLICATIONS

CHIP shRNA (h) Lentiviral Particles is recommended for the inhibition of CHIP expression in human 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

CHIP (G-2): sc-133066 is recommended as a control antibody for monitoring of CHIP 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 CHIP gene expression knockdown using RT-PCR Primer: CHIP (h)-PR: sc-43555-PR (20 μ l, 416 bp). 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.

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