SANTA CRUZ BIOTECHNOLOGY, INC.

Hip1r siRNA (m): sc-145969

BACKGROUND

Huntington disease (HD) is an inherited neurodegenerative disorder, which is associated with the expansion of a polyglutamine tract, greater than 35 repeats, in the HD gene product huntingtin. Hip1 (huntingtin-interacting protein 1) and its related protein Hip1r are multi-domain proteins that form homodimers, interact with inositol lipids, clathrin and actin via their epsin N-terminal homology (ENTH) domains, and are involved in vesicular trafficking. Double Hip1 and Hip1r knockout (DKO) mice are dwarfed, afflicted with severe vertebral defects and die in early adulthood. Single Hip1 or Hip1r knockouts do not display these phenotypes suggesting compensatory roles for Hip1 and Hip1r. Specifically, Hip1r is a component of clathrin-coated pits and vesicles that may link the endocytic machinery to the actin cytoskeleton.

REFERENCES

1. Seki, N., et al. 1998. Cloning, expression analysis, and chromosomal localization of Hip1r, an isolog of huntingtin interacting protein (Hip1). J. Hum. Genet. 43: 268-271.
2. Engqvist-Goldstein, A.E., et al. 2001. The actin-binding protein Hip1r associates with clathrin during early stages of endocytosis and promotes clathrin assembly *in vitro*. J. Cell Biol. 154: 1209-1223.
3. Hyun, T.S., et al. 2004. Hip1 and Hip1r stabilize receptor tyrosine kinases and bind 3-phosphoinositides via epsin N-terminal homology domains. J. Biol. Chem. 279: 14294-14306.
4. Hyun, T.S., et al. 2004. Hip1-related mutant mice grow and develop normally but have accelerated spinal abnormalities and dwarfism in the absence of Hip1. Mol. Cell. Biol. 24: 4329-4340.
5. Brett, T.J., et al. 2006. Structural definition of the F-actin-binding THATCH domain from Hip1r. Nat. Struct. Mol. Biol. 13: 121-130.
6. Bradley, S.V., et al. 2007. Degenerative phenotypes caused by the combined deficiency of murine Hip1 and Hip1r are rescued by human Hip1. Hum. Mol. Genet. 16: 1279-1292.
7. Wilbur, J.D., et al. 2008. Actin binding by Hip1 (huntingtin-interacting protein 1) and Hip1r (Hip1-related protein) is regulated by clathrin light chain. J. Biol. Chem. 283: 32870-32879.

CHROMOSOMAL LOCATION

Genetic locus: Hip1r (mouse) mapping to 5 F.

PRODUCT

Hip1r 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hip1r shRNA Plasmid (m): sc-145969-SH and Hip1r shRNA (m) Lentiviral Particles: sc-145969-V as alternate gene silencing products.

For independent verification of Hip1r (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-145969A, sc-145969B and sc-145969C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Hip1r siRNA (m) is recommended for the inhibition of Hip1r 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 µM in 66 µl. 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

HIP12 (1C5): sc-58533 is recommended as a control antibody for monitoring of Hip1r 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Hip1r gene expression knockdown using RT-PCR Primer: Hip1r (m)-PR: sc-145969-PR (20 µl). 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.