SANTA CRUZ BIOTECHNOLOGY, INC.

HRI siRNA (h): sc-39052

BACKGROUND

HRI (heme-regulated inhibitor kinase) phosphorylates the α subunit of eIF2α kinase, which plays an important role in translational regulation during heme deficiency. HRI is activated in response to a number of environmental conditions, including heme deficiency, heat shock, and oxidative stress. Auto-phosphorylation is essential for the activation of HRI, which causes an arrest of initiation of protein synthesis. Both HSP 90 and HSC 70 are necessary for all stress-induced HRI activation. Furthermore, HSC 70 is required for the folding and transformation of HRI into an active kinase and is subsequently required to negatively attenuate the activation of transformed HRI. Both the N-terminus and the kinase insertion domain, which are unique to HRI, are involved in the heme binding and the heme regulation of HRI. The human HRI gene maps to chromosome 7p22.1 and encodes a 630 amino acid protein expressed mainly in erythroid cells.

REFERENCES

1. Fagard, R. and London, I.M. 1981. Relationship between phosphorylation and activity of heme-regulated eukaryotic initiation factor 2α kinase. Proc. Natl. Acad. Sci. USA 78: 866-870.
2. Matts, R.L., et al. 1991. Toxic heavy metal ions activate the heme-regulated eukaryotic initiation factor-2α kinase by inhibiting the capacity of hemin-supplemented reticulocyte lysates to reduce disulfide bonds. J. Biol. Chem. 266: 12695-12702.
3. Uma, S., et al. 1999. Dual role for HSC 70 in the biogenesis and regulation of the heme-regulated kinase of the α subunit of eukaryotic translation initiation factor 2. Mol. Cell. Biol. 19: 5861-5871.

CHROMOSOMAL LOCATION

Genetic locus: EIF2AK1 (human) mapping to 7p22.1.

PRODUCT

HRI siRNA (h) 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 HRI shRNA Plasmid (h): sc-39052-SH and HRI shRNA (h) Lentiviral Particles: sc-39052-V as alternate gene silencing products.

For independent verification of HRI (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-39052A, sc-39052B and sc-39052C.

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

HRI siRNA (h) is recommended for the inhibition of HRI expression in human 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

HRI (D-12): sc-365239 is recommended as a control antibody for monitoring of HRI 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 HRI gene expression knockdown using RT-PCR Primer: HRI (h)-PR: sc-39052-PR (20 µl, 566 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Fu, X., et al. 2016. Malonate induces the assembly of cytoplasmic stress granules. FEBS Lett. 590: 22-33.
2. Timalsina, S., et al. 2018. Chemical compounds that suppress hypoxia-induced stress granule formation enhance cancer drug sensitivity of human cervical cancer HeLa cells. J. Biochem. 164: 381-391.

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.