SANTA CRUZ BIOTECHNOLOGY, INC.

ARIH1 siRNA (h): sc-90171

BACKGROUND

ARIH1 (ariadne homolog), also known as ubiquitin conjugating enzyme E2 binding protein 1, ARI, HARI, HHARI (human homolog of *Drosophila* ariadne), MOP-6 (monocyte protein 6) or UBCH7BP (UBCH7 binding protein), is a 557 amino acid cytoplasmic protein. Expressed in a wide variety of tissues, ARIH1 contains two RING-type zinc fingers and one IBR (in-between RING fingers)-type domain. ARIH1 is believed to be involved in protein degradation and protein translation. ARIH1 interacts with UBCH7 and is thought to function as an E3 ubiquitin-protein ligase (or as a component of an E3 complex) that, characteristic of E3 ligase proteins, accepts ubiquitin (in the form of a thio-ester) from an E2 ubiquitin-conjugating enzyme (UBCH7) and transfers that ubiquitin residue to substrates targeted for degradation. Specifically, ARIH1 interacts with and polyubiquitylates eIF4E2, thereby targeting it for proteasomal degradation.

REFERENCES

1. Moynihan, T.P., Ardley, H.C., Nuber, U., Rose, S.A., Jones, P.F., Markham, A.F., Scheffner, M. and Robinson, P.A. 1999. The ubiquitin-conjugating enzymes UBCH7 and UBCH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. J. Biol. Chem. 274: 30963-30968.
2. Tan, N.G., Ardley, H.C., Rose, S.A., Leek, J.P., Markham, A.F. and Robinson, P.A. 2000. Characterisation of the human and mouse orthologues of the *Drosophila* ariadne gene. Cytogenet. Cell Genet. 90: 242-245.
3. Ardley, H.C., Tan, N.G., Rose, S.A., Markham, A.F. and Robinson, P.A. 2001. Features of the Parkin/ariadne-like ubiquitin ligase, HHARI, that regulate its interaction with the ubiquitin-conjugating enzyme, UBCH7. J. Biol. Chem. 276: 19640-19647.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605624. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Tan, N.G., Ardley, H.C., Scott, G.B., Rose, S.A., Markham, A.F. and Robinson, P.A. 2003. Human homologue of ariadne promotes the ubiquitylation of translation initiation factor 4E homologous protein, 4EHP. FEBS Lett. 554: 501-504.

CHROMOSOMAL LOCATION

Genetic locus: ARIH1 (human) mapping to 15q24.1.

PRODUCT

ARIH1 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 ARIH1 shRNA Plasmid (h): sc-90171-SH and ARIH1 shRNA (h) Lentiviral Particles: sc-90171-V as alternate gene silencing products.

For independent verification of ARIH1 (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-90171A, sc-90171B and sc-90171C.

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

ARIH1 siRNA (h) is recommended for the inhibition of ARIH1 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

ARIH1 (C-7): sc-514551 is recommended as a control antibody for monitoring of ARIH1 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 ARIH1 gene expression knockdown using RT-PCR Primer: ARIH1 (h)-PR: sc-90171-PR (20 µl, 431 bp). 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.