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HIRA shRNA (h) Lentiviral Particles: sc-43836-V

BACKGROUND

HIRA is the human homolog of yeast Hir1p and Hir2p and is a widely expressed nuclear protein involved in cell cycle regulation. Specifically, HIRA is essential during development, possibly through the control of specific gene transcription programs. During development, HIRA is highly expressed in regions that contain neural crest cells. Cyclin dependent kinase 2 (Cdk-2) and Cyclin A bind with HIRA at an RXL motif which results in phosphorylation of the substrate at Thr 555. Ectopic expression of HIRA results in cell cycle arrest in S phase. HIRA also contains seven copies of a WD repeat and exhibits histone binding properties, suggesting that it may function as a regulator of histone gene expression. The gene encoding the 1,017 amino acid HIRA maps to human chromosome 22q11.21, an area known to be the critical region of DiGeorge Syndrome (DGS). DGS is a congenital disease characterized by defects in tissues and organs, whose development depends on cell populations derived from the neural crest.

REFERENCES

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- Lamour, V., et al. 1995. A human homolog of the *S. cerevisiae* HIR1 and HIR2 transcriptional repressors cloned from the DiGeorge syndrome critical region. *Hum. Mol. Genet.* 5: 791-799.
- Lorain, S., et al. 1996. Structural Organization of the WD repeat protein-encoding gene HIRA in the DiGeorge syndrome critical region of human chromosome 22. *Genome Res.* 1: 43-50.
- Farrell, M.J., et al. 1999. HIRA, a DiGeorge syndrome candidate gene, is required for cardiac outflow tract septation. *J. Clin. Invest.* 12: 1509-1517.
- De Lucia, F., et al. 2001. Subnuclear localization and mitotic phosphorylation of HIRA, the human homologue of *Saccharomyces cerevisiae* transcriptional regulators Hir1p/Hir2p. *Biochem. J.* 358: 447-455.

CHROMOSOMAL LOCATION

Genetic locus: HIRA (human) mapping to 22q11.21.

PRODUCT

HIRA 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 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 HIRA siRNA (h): sc-43836 and HIRA shRNA Plasmid (h): sc-43836-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

HIRA shRNA (h) Lentiviral Particles is recommended for the inhibition of HIRA 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

HIRA (374C6a): sc-130636 is recommended as a control antibody for monitoring of HIRA 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 HIRA gene expression knockdown using RT-PCR Primer: HIRA (h)-PR: sc-43836-PR (20 μ 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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