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GAK shRNA (h) Lentiviral Particles: sc-43791-V

BACKGROUND

Cyclins are the regulatory subunits of Cdc2 p34 and related cyclin-dependent kinases (Cdks) which play critical roles in the control of cell cycle progression. The catalytic subunit for cyclin A and B is Cdc2 p34 kinase. The Cdc2-cyclin B complex controls the G₂ to M transition whereas Cdc2-cyclin A regulates S phase progression. The G₁ to S transition, however, appears to be controlled by the G₁ cyclins. Cyclin D1 accumulates during G₁ and associates with Cdk2, Cdk4 and Cdk5. Cyclin E and Cdk2 interact during the G₁ to S transition. Cyclin G contains a typical N terminal cyclin box and a carboxy terminal domain sequence homologous to the tyrosine phosphorylation site of the epidermal growth factor receptor. Cyclin G expression is induced within three hours after growth stimulation and remains elevated with no apparent cell cycle dependency. A serine/threonine kinase, designated GAK for cyclin G associated kinase, has been identified. GAK has been shown to bind directly to cyclin G and to co-immunoprecipitate with Cdk5, which also associates with cyclin G.

REFERENCES

1. Pines, J., et al. 1990. Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. *Nature* 346: 760-763.
2. Fang, F., et al. 1991. Evidence that the G₁-S and G₂-M transitions are controlled by different Cdc2 proteins in higher eukaryotes. *Cell* 66: 731-742.
3. Koff, A., et al. 1991. Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. *Cell* 66: 1217-1228.
4. Girard, F., et al. 1991. Cyclin A is required for the onset of DNA replication in mammalian fibroblasts. *Cell* 67: 1169-1179.
5. Matsushime, H., et al. 1992. Identification and properties of an atypical catalytic subunit (p34PSK-J3/Cdk4) for mammalian D type G₁ cyclins. *Cell* 71: 323-334.
6. Xiong, Y., et al. 1992. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* 71: 505-514.
7. Tamura, K., et al. 1993. Cyclin G: a new mammalian cyclin with homology to fission yeast Cig1. *Oncogene* 8: 2113-2118.

CHROMOSOMAL LOCATION

Genetic locus: GAK (human) mapping to 4p16.3.

PRODUCT

GAK 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 µl frozen stock containing 1.0 x 10⁶ 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 GAK siRNA (h): sc-43791 and GAK shRNA Plasmid (h): sc-43791-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

GAK shRNA (h) Lentiviral Particles is recommended for the inhibition of GAK expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

GAK (D-2): sc-137053 is recommended as a control antibody for monitoring of GAK 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 GAK gene expression knockdown using RT-PCR Primer: GAK (h)-PR: sc-43791-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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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