SANTA CRUZ BIOTECHNOLOGY, INC.

GAK siRNA (m): sc-63301

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 G_2 to M transition whereas Cdc2-cyclin A regulates S phase progression. The G_1 to S transition, however, appears to be controlled by the G_1 cyclins. Cyclin D1 accumulates during G_1 and associates with Cdk2, Cdk4 and Cdk5. Cyclin E and Cdk2 interact during the G_1 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 G_1-S and G_2-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 G_1 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.
8. Kanaoka, Y., et al. 1997. GAK: a cyclin G associated kinase contains a tensin/auxilin-like domain. FEBS Lett. 402: 73-80.

CHROMOSOMAL LOCATION

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

PRODUCT

GAK 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 GAK shRNA Plasmid (m): sc-63301-SH and GAK shRNA (m) Lentiviral Particles: sc-63301-V as alternate gene silencing products.

For independent verification of GAK (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-63301A, sc-63301B and sc-63301C.

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

GAK siRNA (m) is recommended for the inhibition of GAK 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

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 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 GAK gene expression knockdown using RT-PCR Primer: GAK (m)-PR: sc-63301-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.