SANTA CRUZ BIOTECHNOLOGY, INC.

NIPA siRNA (m): sc-61198

BACKGROUND

Entry into mitosis is essentially driven by cyclin B1 which is located in the cytoplasm throughout interphase, but accumulates in the nucleus just before mitosis occurs. Nuclear Interaction Partner of ALK (NIPA) plays a critical role in cyclin B1 regulation. NIPA is normally phosphorylated during G_2 and M phases, resulting in an accumulation of cyclin B1. When NIPA sheds its attached phosphate, it binds to SCF to form the SCFNIPA complex, a member of the E3 ubiquitin ligases, which ubiquitinates cyclin B1, thereby targeting it to the proteosome for degradation. Therefore, the accumulation of cyclin B1 is due to the inability of phosphorylated NIPA to bind to the molecule SCF, thereby preventing the degradation of cyclin B1. An absence of NIPA causes cyclin B1 to accumulate abnormally, leading to premature mitotic entry, loss of checkpoint control, and genomic instability which are all associated with cancer. The phosphorylated form of NIPA may also be involved in apoptotic signaling pathways.

REFERENCES

1. Lamant, L., et al. 1999. A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood 93: 3088-3095.
2. Golan, A., et al. 2002. The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly by protein kinases Cdk1-cyclin B and Plk. J. Biol. Chem. 277: 15552-15557.
3. Ayad, N.G., et al. 2003. Tome-1, a trigger of mitotic entry, is degraded during G_1 via the APC. Cell 113: 101-113.
4. Bassermann, F., et al. 2005. NIPA defines an SCF-type mammalian E3 ligase that regulates mitotic entry. Cell 122: 45-57.
5. Bassermann, F., et al. 2005. Mitotic entry: a matter of oscillating destruction. Cell Cycle 4: 1515-1517.

CHROMOSOMAL LOCATION

Genetic locus: Zc3hc1 (mouse) mapping to 6 A3.3.

PRODUCT

NIPA 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 NIPA shRNA Plasmid (m): sc-61198-SH and NIPA shRNA (m) Lentiviral Particles: sc-61198-V as alternate gene silencing products.

For independent verification of NIPA (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-61198A, sc-61198B and sc-61198C.

PROTOCOLS

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

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

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

NIPA (A-12): sc-514368 is recommended as a control antibody for monitoring of NIPA 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 NIPA gene expression knockdown using RT-PCR Primer: NIPA (m)-PR: sc-61198-PR (20 µl). 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.