

Skp2 p45 siRNA (r): sc-270333

BACKGROUND

The critical role that the family of regulatory proteins known as cyclins plays in eukaryotic cell cycle regulation is well established. The best characterized cyclin complex is the mitotic cyclin B/Cdc2 p34 kinase, the active component of MPF (maturation promoting factor). Cyclin A accumulates prior to cyclin B in the cell cycle, appears to be involved in control of S phase and has been shown to associate with cyclin dependent kinase-2 (Cdk2). In addition, cyclin A has been implicated in cell transformation and is found in complexes with E1A, transcription factors DP-1 and E2F and retino-blastoma protein p110. Two cyclin A-Cdk2 complex binding proteins, Skp1 p19 and Skp2 p45, have been described. Although the Skps (S phase kinase-associated proteins) associate with the active cyclin A-Cdk2 complex, they do not exhibit any regulatory effects on the complex. Abolition of Skp2 p45 function by either microinjection of anti-p45 antibodies or addition of antisense oligonucleotides prevents entry into S phase of both normal and transformed cells.

REFERENCES

1. Draetta, G., et al. 1989. Cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* 56: 829-838.
2. Giordano, A., et al. 1989. A 60 kd Cdc2-associated polypeptide complexes with the E1A proteins in adenovirus-infected cells. *Cell* 58: 981-990.
3. Wang, J., et al. 1990. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* 343: 555-557.
4. Pines, J., et al. 1990. Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. *Nature* 346: 760-763.
5. Bandara, L.R., et al. 1991. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. *Nature* 352: 249-251.
6. Williams, R.T., et al. 1992. Co-purification of p34cdc2/p58 cyclin A proline-directed protein kinase and the retinoblastoma tumor susceptibility gene product: interaction of an oncogenic serine/threonine protein kinase with a tumor-suppressor protein. *Oncogene* 7: 423-432.
7. Lees, E., et al. 1992. Cyclin E/Cdk2 and cyclin A/Cdk2 kinases associate with p107 and E2F in a temporally distinct manner. *Genes Dev.* 6: 1874-1885.

CHROMOSOMAL LOCATION

Genetic locus: Skp2 (rat) mapping to 2q16.

PRODUCT

Skp2 p45 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Skp2 p45 shRNA Plasmid (r): sc-270333-SH and Skp2 p45 shRNA (r) Lentiviral Particles: sc-270333-V as alternate gene silencing products.

For independent verification of Skp2 p45 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270333A, sc-270333B and sc-270333C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Skp2 p45 siRNA (r) is recommended for the inhibition of Skp2 p45 expression in rat 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 μ M in 66 μ l. 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

Skp2 p45 (A-2): sc-74477 is recommended as a control antibody for monitoring of Skp2 p45 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Skp2 p45 gene expression knockdown using RT-PCR Primer: Skp2 p45 (r)-PR: sc-270333-PR (20 μ l). 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.