SANTA CRUZ BIOTECHNOLOGY, INC.

Skp2 p45 siRNA (h): sc-36499

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 retinoblastoma 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. Pines, J., et al. 1990. Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. Nature 346: 760-763.

CHROMOSOMAL LOCATION

Genetic locus: SKP2 (human) mapping to 5p13.2.

PRODUCT

Skp2 p45 siRNA (h) 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 Skp2 p45 shRNA Plasmid (h): sc-36499-SH and Skp2 p45 shRNA (h) Lentiviral Particles: sc-36499-V as alternate gene silencing products.

For independent verification of Skp2 p45 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36499A, sc-36499B and sc-36499C.

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

Skp2 p45 siRNA (h) is recommended for the inhibition of Skp2 p45 expression in human 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

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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Skp2 p45 gene expression knockdown using RT-PCR Primer: Skp2 p45 (h)-PR: sc-36499-PR (20 µl, 429 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Huang, H.C., et al. 2008. EGCG stabilizes p27 Kip1 in E2-stimulated MCF7 cells through down-regulation of the Skp2 protein. Endocrinology 149: 5972-5983.
2. Pernicová, Z., et al. 2011. Androgen depletion induces senescence in prostate cancer cells through down-regulation of Skp2. Neoplasia 13: 526-536.
3. Huang, K.T., et al. 2012. Estrogen and progesterone regulate p27 Kip1 levels via the ubiquitin-proteasome system: pathogenic and therapeutic implications for endometrial cancer. PLoS ONE 7: e46072.
4. Qi, M., et al. 2015. Inhibition of S-phase kinase-associated protein 2-mediated p27 degradation suppresses tumorigenesis and the progression of hepatocellular carcinoma. Mol. Med. Rep. 11: 3934-3940.
5. Thacker, G., et al. 2016. Skp2 inhibits osteogenesis by promoting ubiquitin-proteasome degradation of RUNX2. Biochim. Biophys. Acta 1863: 510-519.
6. Cheng, Y., et al. 2017. A novel read-through transcript JMJD7-PLA2G4B regulates head and neck squamous cell carcinoma cell proliferation and survival. Oncotarget 8: 1972-1982.
7. Thacker, G., et al. 2020. E3 ligase SCF^{SKP2} ubiquitinates and degrades tumor suppressor C/EBPα in acute myeloid leukemia. Life Sci. 257: 118041.

RESEARCH USE

For research use only, not for use in diagnostic procedures.