SANTA CRUZ BIOTECHNOLOGY, INC.

APC4 siRNA (m): sc-29705

BACKGROUND

The anaphase-promoting complex (APC) is composed of more than ten subunits, including APC1, APC2, APC4, APC5, APC7, APC8, APC10, and APC11. The APC acts in a cell-cycle dependent manner to promote the separation of sister chromatids during the transition between metaphase and anaphase in mitosis. APC, or cyclosome, accomplishes this progression through the ubiquitination of mitotic cyclins and other regulatory proteins that are targeted for destruction during cell division. APC is phosphorylated, and thus activated, by protein kinases Cdk1/cyclin B and polo-like kinase (Plk). APC is under tight control by a number of regulatory factors, including CDC20, CDH1 and MAD2. Specifically, CDC20 and CDH1 directly bind to and activate the cyclin-ubiquitination activity of APCs. In contrast, MAD2 inhibits APC by forming a ternary complex with CDC20 and APC, thus preventing APC activation. APC4, also known as ANAPC4, is an 808 amino acid component of the APC.

REFERENCES

1. Jorgensen, P.M., et al. 1998. A subunit of the anaphase-promoting complex is a centromere-associated protein in mammalian cells. Mol. Cell. Biol. 18: 468-476.
2. Page, A.M., et al. 1999. The anaphase-promoting complex: new subunits and regulators. Annu. Rev. Biochem. 68: 583-609.
3. Peters, J.M. 1999. Subunits and substrates of the anaphase-promoting complex. Exp. Cell Res. 248: 339-349.
4. Fang, G., et al. 1999. Control of mitotic transitions by the anaphase-promoting complex. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 354: 1583-1590.
5. Jorgensen, P.M., et al. 2001. Characterisation of the human APC1, the largest subunit of the anaphase-promoting complex. Gene 262: 51-59.

CHROMOSOMAL LOCATION

Genetic locus: Anapc4 (mouse) mapping to 5 C1.

PRODUCT

APC4 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 APC4 shRNA Plasmid (m): sc-29705-SH and APC4 shRNA (m) Lentiviral Particles: sc-29705-V as alternate gene silencing products.

For independent verification of APC4 (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-29705A, sc-29705B and sc-29705C.

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.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

APC4 siRNA (m) is recommended for the inhibition of APC4 expression in mouse cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

APC4 (B-11): sc-514895 is recommended as a control antibody for monitoring of APC4 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 APC4 gene expression knockdown using RT-PCR Primer: APC4 (m)-PR: sc-29705-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.