SANTA CRUZ BIOTECHNOLOGY, INC.

ANAPC2 siRNA (h): sc-77332

BACKGROUND

Comprising more than ten subunits, the anaphase-promoting complex (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 Cdc2/cyclin B and polo-like kinase (Plk). APC is under tight control by a number of regulatory factors, including p55 CDC, E-cadherin and MAD2. Specifically, p55 CDC and E-cadherin directly bind to APC and activate the cyclin-ubiquitination activity of APC. In contrast, MAD2 inhibits APC by forming a ternary complex with p55 CDC and APC and thus preventing APC activation. A heterodimeric complex of either Ubc4 or UbcH10 with ANAPC2 (also known as APC2) and APC11 catalyzes the ubiquitination of human securin and cyclin B1. ANAPC2 contains a C-terminal cullin homology domain that binds both APC11 and UBE2C.

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. Tang, Z., et al. 2001. APC2 cullin protein and APC11 RING protein comprise the minimal ubiquitin ligase module of the anaphase-promoting complex. Mol. Biol. Cell 12: 3839-3851.
6. Golan, A., et al. 2002. The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly activated by protein kinases Cdk1/cyclin B and Plk. J. Biol. Chem. 277: 15552-15557.

CHROMOSOMAL LOCATION

Genetic locus: ANAPC2 (human) mapping to 9q34.3.

PRODUCT

ANAPC2 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 ANAPC2 shRNA Plasmid (h): sc-77332-SH and ANAPC2 shRNA (h) Lentiviral Particles: sc-77332-V as alternate gene silencing products.

For independent verification of ANAPC2 (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-77332A, sc-77332B and sc-77332C.

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

ANAPC2 siRNA (h) is recommended for the inhibition of ANAPC2 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

ANAPC2 (8G2): sc-517022 is recommended as a control antibody for monitoring of ANAPC2 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 ANAPC2 gene expression knockdown using RT-PCR Primer: ANAPC2 (h)-PR: sc-77332-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.

PROTOCOLS

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