

APC8 siRNA (h): sc-37530

BACKGROUND

Composed of 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 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 APC and activate the cyclin-ubiquitination activity of APCs. In contrast, MAD2 inhibits APC by forming a ternary complex with CDC20 and APC and thus preventing APC activation. APC8, also referred to as CDC23, contains nine tetratricopeptide repeat (TPR) units. The TPR is a 34 amino acid sequence that is common to a variety of proteins and is significant because it forms scaffolds to mediate protein-protein interactions. The APC8 gene maps to human chromosome 5q31.2, within the smallest commonly deleted segment in myeloid leukemias.

REFERENCES

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2. Zhao, N., et al. 1998. Protein Human CDC23: cDNA cloning, mapping to 5q31, genomic structure, and evaluation as a candidate tumor suppressor gene in myeloid leukemias. *Genomics* 53: 184-190.
3. Das, A.K., et al. 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO J.* 17: 1192-1199.
4. Page, A.M., et al. 1999. The anaphase-promoting complex: new subunits and regulators. *Annu. Rev. Biochem.* 68: 583-609.
5. Peters, J.M. 1999. Subunits and substrates of the anaphase-promoting complex. *Exp. Cell Res.* 248: 339-349.
6. 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.

CHROMOSOMAL LOCATION

Genetic locus: CDC23 (human) mapping to 5q31.2.

PRODUCT

APC8 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see APC8 shRNA Plasmid (h): sc-37530-SH and APC8 shRNA (h) Lentiviral Particles: sc-37530-V as alternate gene silencing products.

For independent verification of APC8 (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-37530A, sc-37530B and sc-37530C.

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

APC8 siRNA (h) is recommended for the inhibition of APC8 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 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

APC8 (D-7): sc-514006 is recommended as a control antibody for monitoring of APC8 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 APC8 gene expression knockdown using RT-PCR Primer: APC8 (h)-PR: sc-37530-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.