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Cdc6 (h): 293T Lysate: sc-173781

BACKGROUND

Cdc6 is essential for DNA replication. Phosphorylation of Cdc6 is regulated during the cell cycle. *In vitro*, Cdc6 is an excellent substrate for Cdk2, while *in vivo*, Cdc6 is phosphorylated at three sites (Serine 54, Serine 74 and Serine 106). Serine 54, Serine 74 and Serine 106 are also phosphorylated by Cdk2 *in vitro*, which strongly suggests that Cdc6 is an endogenous Cdk substrate. Phosphorylation of Cdc6 by Cdks regulates DNA replication by promoting initiation of DNA replication and, subsequently, by preventing DNA rereplication through nuclear exclusion. Cdc6 is nuclear in G₁, but translocates to the cytoplasm at the start of S phase via CRM1-dependent export. CRM1 binds to its cargo in the nucleus in the presence of a small nuclear GTPase protein, RanGTP. After the RanGTP-CRM1-cargo complex is translocated from the nucleus to the cytoplasm, RanGTP is hydrolyzed to RanGDP, causing the cargo to dissociate from CRM1.

REFERENCES

1. Stade, K., et al. 1997. Exportin 1 (CRM1p) is an essential nuclear export factor. *Cell* 90: 1041-1050.
2. Yan, Z., et al. 1998. Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proc. Natl. Acad. Sci. USA* 95: 3603-3608.
3. Stoeber, K., et al. 1998. Cdc6 protein causes premature entry into S phase in a mammalian cell-free system. *EMBO J.* 17: 7219-7229.
4. Saha, P., et al. 1998. Human Cdc6/Cdc18 associates with Orc1 and cyclin-Cdk and is selectively eliminated from the nucleus at the onset of S phase. *Mol. Cell. Biol.* 18: 2758-2767.
5. Jiang, W., et al. 1999. Multistep regulation of DNA replication by Cdk phosphorylation of HsCdc6. *Proc. Natl. Acad. Sci. USA* 96: 6193-6198.
6. Petersen, B.O., et al. 1999. Phosphorylation of mammalian Cdc6 by cyclin A/Cdk2 regulates its subcellular localization. *EMBO J.* 18: 396-410.

CHROMOSOMAL LOCATION

Genetic locus: CDC6 (human) mapping to 17q21.2.

PRODUCT

Cdc6 (h): 293T Lysate represents a lysate of human Cdc6 transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

RESEARCH USE

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

APPLICATIONS

Cdc6 (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Cdc6 antibodies. Recommended use: 10-20 µl per lane.

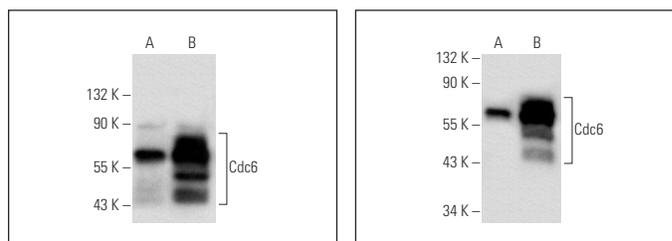
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

Cdc6 (O.T.17): sc-70826 is recommended as a positive control antibody for Western Blot analysis of enhanced human Cdc6 expression in Cdc6 transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

RECOMMENDED SUPPORT REAGENTS

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.

DATA



Cdc6 (180.2): sc-9964. Western blot analysis of Cdc6 expression in non-transfected: sc-117752 (A) and human Cdc6 transfected: sc-173781 (B) 293T whole cell lysates.

Cdc6 (O.T.17): sc-70826. Western blot analysis of Cdc6 expression in non-transfected: sc-117752 (A) and human Cdc6 transfected: sc-173781 (B) 293T whole cell lysates.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

PROTOCOLS

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