

# p-Cdc6 (Ser 74): sc-12921

## 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 Cdk2 regulates DNA replication by promoting initiation of DNA replication and, subsequently, by preventing DNA rereplication through nuclear exclusion. Cdc6 is nuclear in G<sub>1</sub>, 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; Cdc6 (mouse) mapping to 11 D.

## SOURCE

p-Cdc6 (Ser 74) is available as either goat (sc-12921) or rabbit (sc-12921-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing Ser 74 phosphorylated Cdc6 of human origin.

## PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-12921 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

## STORAGE

Store at 4° C, **\*\*DO NOT FREEZE\*\***. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

## APPLICATIONS

p-Cdc6 (Ser 74) is recommended for detection of Ser 74 phosphorylated Cdc6 of mouse and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-Cdc6 (Ser 74) is also recommended for detection of correspondingly phosphorylated Cdc6 in additional species, including equine, canine, bovine and porcine.

Suitable for use as control antibody for Cdc6 siRNA (h): sc-29258, Cdc6 siRNA (m): sc-35046, Cdc6 shRNA Plasmid (h): sc-29258-SH, Cdc6 shRNA Plasmid (m): sc-35046-SH, Cdc6 shRNA (h) Lentiviral Particles: sc-29258-V and Cdc6 shRNA (m) Lentiviral Particles: sc-35046-V.

Molecular Weight of p-Cdc6: 62 kDa.

## RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: for goat primary antibody (sc-12921): use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), for rabbit primary antibody (sc-12921-R): use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent) and Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunofluorescence: for goat primary antibody (sc-12921): use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941, for rabbit primary antibody (sc-12921-R): use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.