

## Anti-CDC6 antibody, rabbit polyclonal, ChIP-Grade

70-133 100  $\mu$ g

**Storage:** Sent at 4°C and upon arrival, spin-down and store at -20°C

**Reactivity:** human, mouse, rat

**Immunogen:** Recombinant GST-human CDC6 (1-326 amino acids) expressed in E. coli

**Validation:** Specificity of reaction has been validated with siRNA in WB (Ref.5 & 7)

### Applications:

1. Western blotting (1/1,000~3,000)
2. Immunoprecipitation (1/200)
3. Chromatin Immuno-Precipitation (assay dependent)
4. Immunofluorescence staining (1/200)

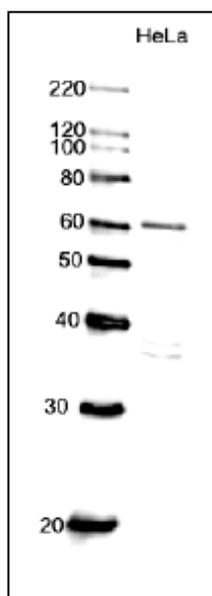
**Form:** 1 mg/ml in PBS with 50% glycerol. Filter-sterilized. Azide and carrier free

**Function:** CDC6 (Cell Division Control Protein 6 homolog) (human; 560 aa, 62.7 kDa) is involved in the initiation of DNA replication. Also participates in checkpoint controls that ensure DNA replication is completed before mitosis is initiated.

**Subcellular location:** The protein is nuclear in G1 and cytoplasmic in S-phase cells.

**Purification:** The antiserum was first adsorbed with GST conjugated agarose column and then the antibody in the pass-through fractions were purified with GST-CDC6 conjugated column.

**Data base Link:** [uniprot/Q99741](http://uniprot.org/Q99741) (CDC6\_HUMAN)

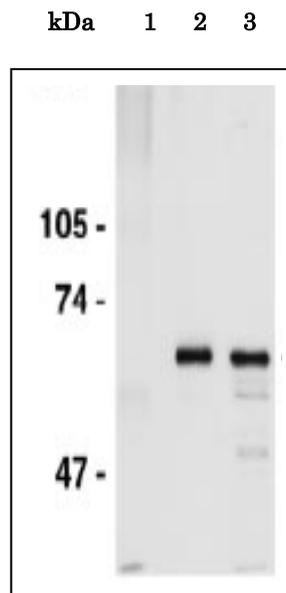


**Fig.1 Western blotting of endogenous CDC6 protein in HeLa cells**

Lane 1; Size marker proteins (kDa)

Lane 2; HeLa cell whole lysate (10  $\mu$ g)

Anti-CDC6 antibody was used at 1/1,000 dilution.



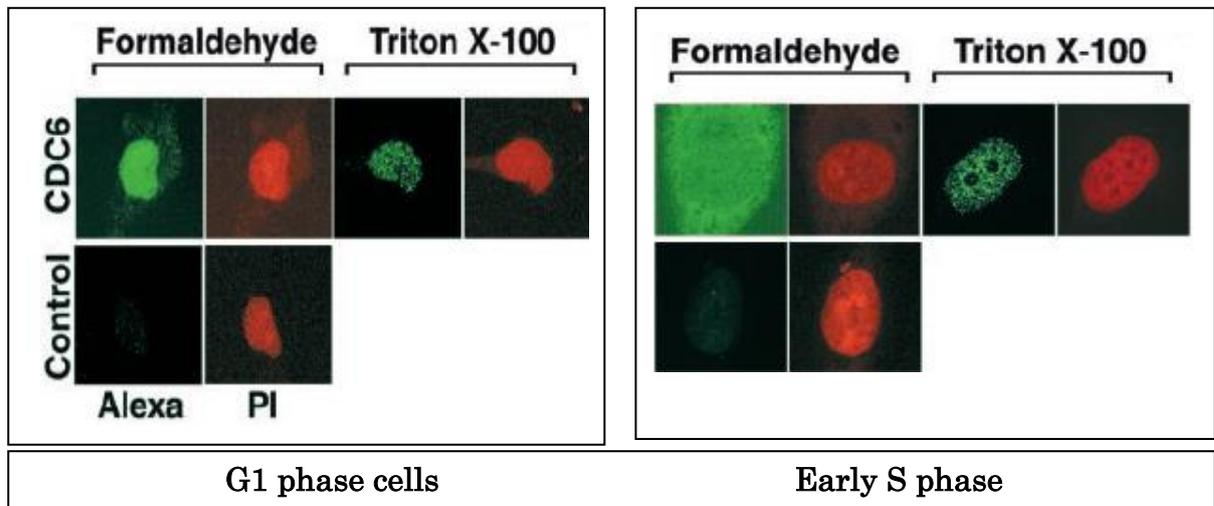
**Fig 2. Immunoprecipitation of CDC6.**

CDC6 protein was precipitated from whole cell lysate of HeLa cells with anti-CDC6 antibody beads and probed by western blotting with anti-CDC6 antibody.

1; Control IP with non-immune IgG

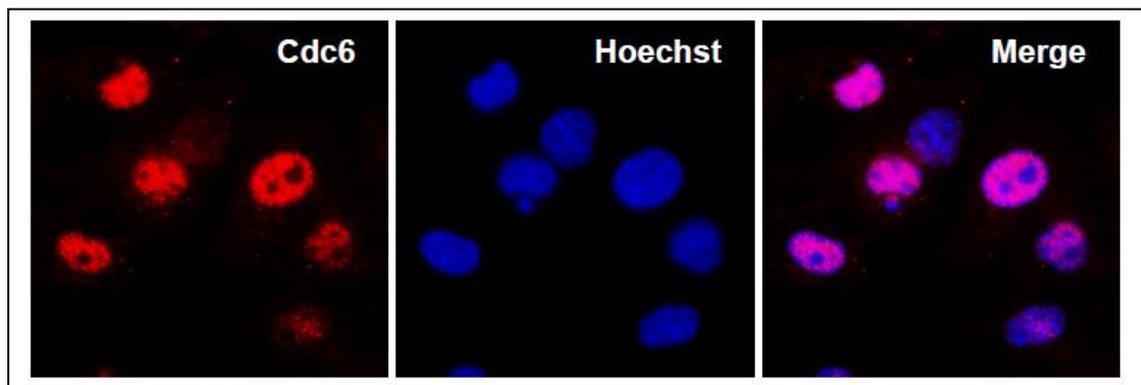
2; Immunoprecipitates with anti-CDC6 antibody

3; Input (whole cell lysate)



**Fig.3 Immunofluorescence staining of CDC6 protein in HeLa cells**

Confocal microscopic analyses of localization of CDC6 proteins in HeLa cells in G<sub>1</sub> or early S phase. Cells in mid to late G<sub>1</sub> phase or hydroxyurea-treated early S phase were fixed with formaldehyde directly or after extraction with Triton X-100 and then immunostained with anti-CDC6 or control non-immune antibody (Alexa;green). The samples were further treated with propidium iodide for DNA staining (PI; red).



**Fig.4 Immunofluorescence staining of CDC6 in MEF cells**

MEFs (mouse embryonic fibroblasts) from embryonic day 14.5 mouse embryos were fixed with 10 % formalin at room temperature (RT) for 10 min, permeabilized with ice-cold methanol on ice for 10 min, and treated with 2 % BSA/PBS at RT for 1h. The cells were reacted with anti-cdc6 antibody (1:200) at 4°C overnight and then with Alexa 555-conjugated goat anti-rabbit IgG antibody (1:1000) at RT for 1h. Chromosomal DNA was counterstained with 3.3 μM Hoechst 33342. Note that CDC6 abundantly localizes in nuclei of the cells at G<sub>1</sub> and early S phase..

**Reference:** This antibody was described in Ref.1 and used in the following publications.

1. Fujita M. et al. (1999) Cell cycle regulation of human CDC6 protein. Intracellular localization, interaction with the human mcm complex, and CDC2 kinase-mediated hyperphosphorylation. [J Biol Chem.](#) 274:25927-32. PMID:10464337, **WB, IP, IF, (human)**
2. Fujita M. et al. (2002) Nuclear organization of DNA replication initiation proteins in mammalian cells. [J Biol Chem.](#) 277:10354-61. PMID: 11779870, **WB, IP, IF, (human)**
3. Tatsumi Y. et al. (2006) Deregulation of Cdt1 induces chromosomal damage without rereplication and leads to chromosomal instability. [J Cell Sci.](#) 119:3128-40. PMID: 16835273. **WB, (human)**
4. Sugimoto N. et al. (2009) Redundant and differential regulation of multiple licensing factors ensures prevention of re-replication in normal human cells. [J Cell Sci.](#) 15::1184-91. PMID:19339550, **WB, (human)**
5. Yoshida K. et al. (2010) CDC6 interaction with ATR regulates activation of a replication checkpoint in higher eukaryotic cells. [J Cell Sci.](#) 123:225-35. PMID:20048340. **WB, IP, (human)**
6. Sugimoto N. et al. (2011) Chromatin remodeler sucrose nonfermenting 2 homolog (SNF2H) is recruited onto DNA replication origins through interaction with Cdc10 protein-dependent transcript 1 (Cdt1) and promotes pre-replication complex formation. [J Biol Chem.](#) 286:39200-10. PMID:21937426. **WB, (human)**
7. Sugimoto N et al. (2015) Cdt1-binding protein GRWD1 is a novel histone-binding protein that facilitates MCM loading through its influence on chromatin architecture. [Nucleic Acids Res.](#) 2015 Jul 13;43(12):5898-911. PMID:25990725. **WB, ChIP (human)**