

Anti-CDC6 antibody, rabbit polyclonal, ChIP-Grade

Product code	70-133
Size	100 μg
Storage	-20°C
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol. Filter-sterilized. Azide and carrier free
Purity	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.
Immunogen	Recombinant GST-human CDC6 (1-326 amino acids) expressed in <i>E. coli</i>
Isotype	Rabbit IgG
Reactivity	Human, mouse, rat
Special notes	Validation: Specificity of reaction has been validated with siRNA in WB (Ref.5
	& 7)
Application	1. Western blotting (1/1,000~3,000)
	2. Immunoprecipitation (1/200)
	3. Chromatin Immuno-Precipitation (assay dependent)
	4. Immunofluorescence staining (1/200)
Background	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.
Data Link	uniprot/Q99741 (CDC6_HUMAN)
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC	

PROCEDURES. NOT FOR MILITARY USE.



Data Images: 70-133 Anti-CDC6 antibody, rabbit polyclonal, ChIP-Grade

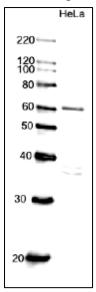


Fig.1 Western blotting of endogenous CDC6 protein in HeLa cells

Lane 1; Size marker proteins (kDa)

Lane 2; HeLa cell whole lysate (10 µg)

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

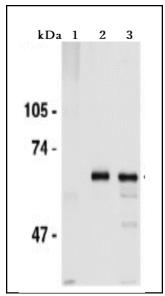


Fig 2. Immunoprecipitation of CDC6.

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)



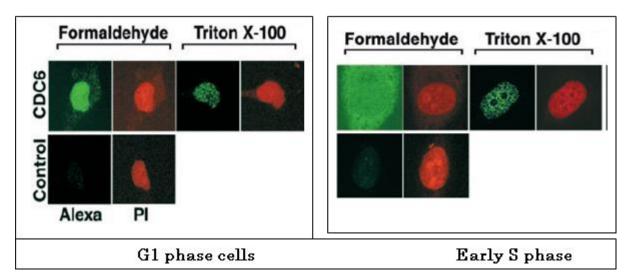


Fig.3 Immunofluorescence staining of CDC6 protein in HeLa cells

Confocal microscopic analyses of localization of CDC6 proteins in HeLa cells in G₁ or early S phase. Cells in mid to late G₁phase 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).

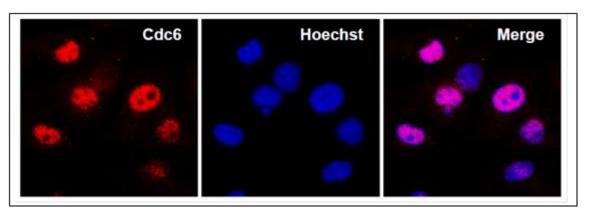


Fig.4 Immunofluorescence staining of CDC6 in MEF cells

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 μ M Hoechst 33342. Note that CDC6 abundantly localizes in nuclei of the cells at G1 and early S phase.

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

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. <u>J Biol Chem.</u> 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. <u>J Biol Chem.</u> 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. <u>J Cell Sci.</u> 119:3128-40. PMID: 16835273. **WB, (human)**
- Sugimoto N. et al. (2009) Redundant and differential regulation of multiple licensing factors ensures prevention of re-replication in normal human cells. <u>J Cell Sci.</u> 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. <u>J Biol Chem.</u> 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. <u>Nucleic Acids Res.</u> 2015 Jul 13;43(12):5898-911. PMID:25990725. **WB, ChIP (human)**