



POLYCLONAL ANTIBODY

For research use only. Not for clinical diagnosis.

Catalog No. 70-115EX

Anti CDT2 antibody

Key words; Ubiquitination, Protein degradation, Cdt1, CDKN1A/p21(CIP1), Cell cycle, DNA damage response, ATR, Phosphorylation, Translesion DNA synthesis

Function; CDT2 (human, 730 aa, 79.5 kDa) is substrate-specific adapter of a DCX (DDB1-CUL4-X-box) E3 ubiquitin-protein ligase complex required for cell cycle control, DNA damage response and translesion DNA synthesis. The DCX(DTL) complex, also named CRL4(CDT2) complex, mediates the polyubiquitination and subsequent degradation of CDT1 and CDKN1A/p21(CIP1). CDT1 degradation in response to DNA damage is necessary to ensure proper cell cycle regulation of DNA replication. CDKN1A/p21(CIP1) degradation during S phase or following UV irradiation is essential to control replication licensing. Most substrates require their interaction with PCNA for their polyubiquitination: substrates interact with PCNA via their PIP-box, and those containing the 'K+4' motif in the PIP box, recruit the DCX(DTL) complex, leading to their degradation. In undamaged proliferating cells, the DCX(DTL) complex also promotes the 'Lys-164' monoubiquitination of PCNA, thereby being involved in PCNA-dependent translesion DNA synthesis. CDT2 is activated by checkpoint kinase ATR following DNA damage.

Product type	Primary antibodies
Host	Rabbit
Source	Serum
Form	Rabbit polyclonal antiserum added with 0.05 % sodium azide.
Volume	100 µL
Concentration	
Specificity	
Antigen	E. coli expressed and purified 6×His-tagged C-terminal 150 amino acids of human Cdt2.

Application notes	<ol style="list-style-type: none">1. Western blotting (1/2,000 dilution).2. Immunoprecipitation (1/100~1/200 dilution)3. Immunofluorescence staining (1/100~1/1,000)4. FACS (assay dependent). <p>Other applications have not been tested. Optimal dilutions/concentrations should be determined by the end user.</p>
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Data Link	SwissProt: Q9NZJ0 Human Entrez Gene: 51514 Human
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Cross reactivity	Human and mouse Cdt2 proteins. Not tested in other species
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Storage	Sent at 4°C or -20°C. Store at -20°C.
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References	1. Nishitani H. et al. (2008) CDK Inhibitor p21 Is Degraded by a Proliferating Cell Nuclear Antigen-coupled Cul4-DDB1 ^{Cdt2} Pathway during S Phase and after UV Irradiation. J.Biol.Chem. 283: 29045-29052. WB
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Inspiration for Life Science

Anti-CDT2 antibody

(This product was described in Ref.1 and used in the following publications).

2. Ishii T. et al. (2010) Proliferating cell nuclear antigen-dependent rapid recruitment of Cdt1 and CRL4Cdt2 at DNA-damaged sites after UV irradiation in HeLa cells. J Biol Chem. 285:41993-42000. **WB, IF, FACS**
 3. Roukos V. et al. (2011) Dynamic recruitment of licensing factor Cdt1 to sites of DNA damage. J. Cell Science 124: 422-434. **IF**
 4. Sakaguchi H. et al. (2012) Checkpoint Kinase ATR Phosphorylates Cdt2, a Substrate Receptor of CRL4 Ubiquitin Ligase, and Promotes the Degradation of Cdt1 following UV Irradiation. PLoS ONE 7(9): e46480. **WB**
 5. Shiomi Y. et al. (2012) Two Different Replication Factor C Proteins, Ctf18 and RFC1, Separately Control PCNA-CRL4^{Cdt2}-Mediated Cdt1 Proteolysis during S Phase and following UV Irradiation. Mol. Cell. Biol. 32: 2279-2288. **WB, IF**
- Nozawa R.S et al (2010) Human POGZ modulates dissociation of HP1alpha from mitotic chromosome arms through Aurora B activation. Nat. Cell Biol. 12:719-727
[PubMed: 20562864](https://pubmed.ncbi.nlm.nih.gov/20562864/)

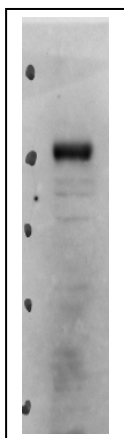


Fig.1. Identification of Cdt2 protein in whole cell extract of HeLa cells by western blotting. With cell extract of 20 µg protein, endogenous level of Cdt2 protein was detected with the antibody at 1/2,000 dilution. Secondary antibody was HRP-conjugated goat anti-rabbit IgG antibody at 1/20,000.

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Anti-CDT2 antibody

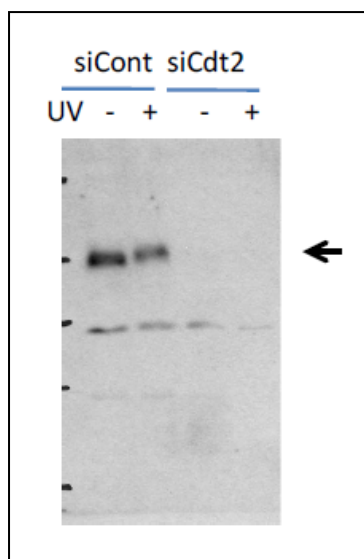


Fig.2. Inhibition of Cdt2 protein synthesis by Cdt2

siRNA introduced into HeLa cells. siCont is control siRNA unrelated to Cdt2. siCdt2 is Cdt2 siRNA.

Cdt2 is phosphorylated after UV irradiation as shown by the band shift-up in irradiated sample (UV +).

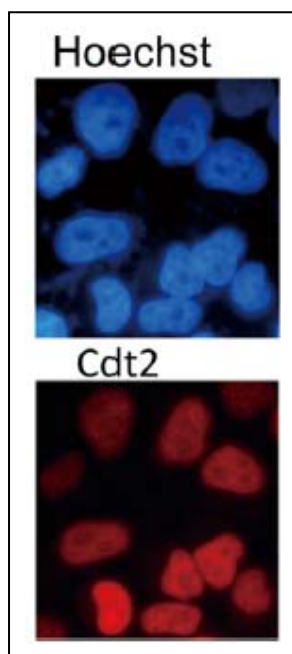


Fig. 3. Immunofluorescence staining of Cdt2 protein in growing HeLa cells with the antibody.

Asynchronously growing HeLa cells were stained with Hoechst 33258 for DNA and with the antibody for Cdt2 protein. The cells were fixed in 4% paraformaldehyde for 10 min, permeabilized in 0.25% (v/v) Triton X-100 in phosphate-buffered saline (PBS), and reacted with the primary antibody. Alexa-592 conjugated goat anti-rabbit IgG was used as secondary antibody. Cdt2 protein is localized in nuclei at all cell stages.

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