

Anti- CDT2 / DTL antibody, rabbit serum

| Product code | 70-115 |
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| Size | 100µl |
| Storage | Store at 4° C for short term. For long term storage store at -20° C. |
| | Aliquot to avoid repeated freezing thawing. |
| Concentration | N/A |
| Buffer | 0.05 % sodium azide |
| Purity | Rabbit antiserum |
| Immunogen | E. coli expressed and purified 6×His-tagged C-terminal 150 amino acids of |
| | human Cdt2 |
| Isotype | Rabbit IgG |
| Reactivity | Human and mouse, rat, hamster |
| Special notes | Specificity was validated by western blotting with siRNA (Fig.2) |
| Application | 1. Western blotting (1/200-1/2,000 dilution). |
| | 2. Immunoprecipitation (assay dependent) |
| | 3. Immunofluorescence staining (1/100~1/1,000 dilution) |
| | 4. Flow Cytometry (assay dependent). |
| | Other applications have not been tested. |
| Background | 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. |
| Data Link | UniProtKB <u>Q9NZJ0</u> Human <u>Entrez Gene: 51514</u> Human |
| Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC | |
| PROCEDURES. NOT FOR MILITARY USE. | |

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Data Images: 70-117 Anti- CDT2 / DTL antibody, rabbit serum

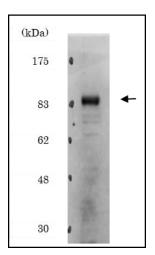


Fig.1. Identification of Cdt2 protein in whole cell extract of HeLa cells by western blotting. With cell extract of 20 μ g protein, endogenous level of Cdt2 protein was detected with the antibody at 1/2,000 dilution. Secondary antibody was HRP-conjugated goat anti-rabbit IgG antibody at 1/20,000.

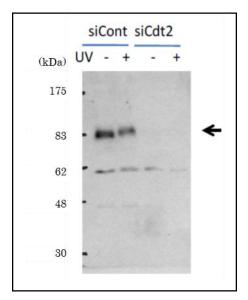


Fig.2. Inhibition of Cdt2 protein synthesis by Cdt2 siRNA introduced into HeLa cells. siCont is control siRNA unrelated to Cdt2. siCdt2 is Cdt2 specific siRNA.

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

References: This product was described in Ref.1 and used in the following publications.

- Nishitani H. et al. (2008) CDK Inhibitor p21 Is Degraded by a Proliferating Cell Nuclear Antigencoupled Cul4-DDB1^{Cdt2} Pathway during S Phase and after UV Irradiation. J.Biol.Chem. 283: 29045-29052. WB <u>Link</u>
- 2. Ishii T. et al. (2010) Proliferating cell nuclear antigen-dependent rapid recruitment of Cdt1 and

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CRL4Cdt2 at DNA-damaged sites after UV irradiation in HeLa cells. J Biol Chem. 285:41993-42000. **WB, IF, FACS** Link

- Roukos V. et al. (2011) Dynamic recruitment of licensing factor Cdt1 to sites of DNA damage. J. Cell Science 124: 422-434. IF <u>Link</u>
- 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 Link
- 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 Link