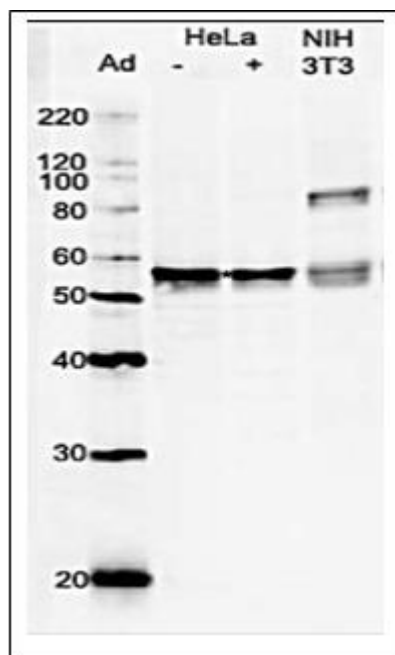


## Anti-GRWD1 antibody, rabbit polyclonal

<b>Product code</b>	70-130
<b>Size</b>	100 µg
<b>Storage</b>	-20°C
<b>Concentration</b>	1.0 mg/ml
<b>Buffer</b>	PBS <sup>-</sup> with 50% glycerol
<b>Purity</b>	Purified IgG fraction with protein A from rabbit antiserum absorbed with GST-agarose column
<b>Immunogen</b>	Purified GST-GRWD1 (human, full-length) expressed in <i>E. coli</i> .
<b>Isotype</b>	Rabbit IgG
<b>Reactivity</b>	Specific to GST and GST-tagged proteins
<b>Special notes</b>	Validated for Western Blotting by siRNA.
<b>Application</b>	<ol style="list-style-type: none"> <li>1. Western blotting (1/1,000~1/3,000 dilution)</li> <li>2. Immunoprecipitation (Assay dependent)</li> <li>3. Chromatin Immuno-Precipitation (Assay dependent)</li> <li>4. Immunofluorescence staining / Immunocytochemistry (1/100~1/1,000 dilution)</li> </ol> <p>KEYWORDS: nucleolar stress response, oncogene, p53, PICT1, RPL11, tumor suppressor</p>
<b>Background</b>	Glutamate-rich WD repeat-containing protein 1 (GRWD1) consists of 446 amino acids with molecular mass of 49.4 kDa. It has been found as a protein which interacts with METTL18 and CDT1 proteins. It has been implicated in regulation of DNA replication and/or in ribosome biogenesis.
<b>Data Link</b>	UniProtKB <a href="#">Q9BQ67</a> (GRWD1_HUMAN) <a href="#">Q810D6</a> (GRWD1_MOUSE)
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

**Data Images:** 70-130 Anti-GRWD1 antibody, rabbit polyclonal



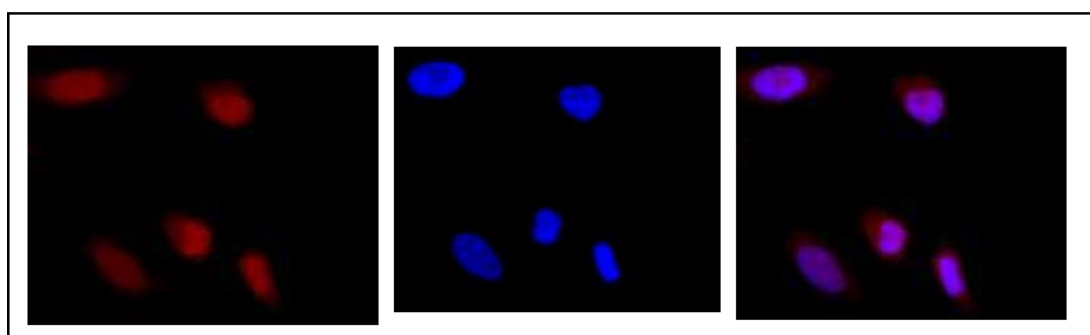
**Fig.1. Identification of GRWD1 proteins in whole cell lysates by western blotting with anti-GRWD1 antibody**

Whole cell lysates of HeLa cells untreated (-) and treated (+) with DNA damaging agent, adriamycin (Ad), and NIH3T3 cells were analyzed by western blotting with anti-GRWD1 antibody at 1/1,000 dilution. The samples were 10  $\mu$ g. Second antibody was HRP-conjugated goat anti-rabbit IgG used at 1/5,000 dilution. The revelation of multiple bands indicates post-translational modification such as phosphorylation. The level of GRWD1 in the cell was not affected by DNA-damaging treatment. The identity of an additional band at ~85 kDa position other than the GRWD1 band in NIH-3T3 cell lysate is not known. The GRWD1 proteins were identified at a position higher (~55 kDa) than expected from the molecular mass of GRWD1 indicated from cDNA sequence (49.4 kDa).

Anti-GRWD1 antibody

DAPI

Merge

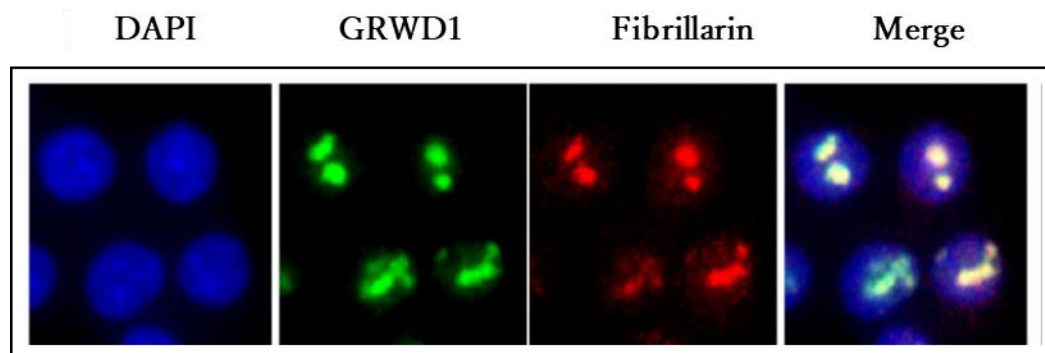


**Fig.2. Immunofluorescence staining of GRWD1 protein in HeLa cells.**

HeLa cells were fixed in 4% paraformaldehyde overnight and permeabilized

in 0.25% TritonX 100 in PBS for 10 min. Anti-GRWD1 antibody was used at 1/1,000 dilution. As second antibody, goat anti-rabbit IgG conjugated with Alex488 was used at 1/5,000 dilution. As a signal enhancer, Can Get Signal Immunostain B (Toyobo, Osaka) was used according to the protocol of the supplier. Nuclei were stained with DAPI.

GRWD1 protein is localized in nuclei.



**Fig.3. Nucleolar localization of GRWD1.**

HCT116 cells were first extracted with Triton X-100 to remove nucleoplasmic proteins, double-immunostained with anti-GRWD1 (green) and anti-fibrillarin (red) antibodies as a marker for nucleoli, and counterstained with DAPI. The anti-GRWD antibody was used at 1/500 dilution and as the second antibody, goat anti-rabbit IgG conjugated with Alex488 was used at 1/2,000 dilution.

**Reference:** This product has been described in Ref.1 and used in the following publication.

- 1.Sugimoto N. et al. Identification of novel human Cdt1-binding proteins by a proteomics approach: proteolytic regulation by APC/CCdh1. [Mol Biol Cell](#). 2008 19(3):1007-21. **WB (human)**
- 2.Sugimoto N. et al. 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, IP, ChIP, IC/IF (human)**
- 3.Aizawa M. et al. Nucleosome assembly and disassembly activity of GRWD1, a novel Cdt1-binding protein that promotes pre-replication complex formation. [Biochim Biophys Acta](#). 2016 Nov;1863(11):2739-2748. PMID:[27552915](#) **WB (human)**
4. Kayama K. et al. GRWD1 negatively regulates p53 via the RPL11-MDM2 pathway and promotes tumorigenesis. [EMBO Rep](#). 2017 Jan;18(1):123-137. PMID:[27856536](#) **WB,IF, IP (human)**