

Anti-GRWD1 antibody, rabbit polyclonal, ChIP grade

70-130 100 ug

Storage: Ship at 4°C and store at -20°C. Do not freeze

Reactivity: Human, mouse and rat. Other species have not been tested.

Validation: Validated for Western Blotting by siRNA.

Immunogen: Purified GST-GRWD1 (human, full-length) expressed in *E. coli*.

Applications:

1. Western blotting (1/1,000~1/3,000 dilution)
2. Immunoprecipitation (Assay dependent)
3. Chromatin Immuno-Precipitation (Assay dependent)
4. Immunofluorescence staining / Immunocytochemistry (1/100~1/1,000 dilution)

KEYWORDS: nucleolar stress response, oncogene, p53, PICT1, RPL11, tumor suppressor

Description: 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.

Purification: The antiserum was first absorbed with GST-agarose column and then the pass-through fraction was affinity-purified with GST-GRWD1 agarose column.

Form: 1 mg/ml in PBS and 50% glycerol. Filter-sterilized. Carrier protein and azide free.

Database link: [uniprot/Q9BQ67](https://www.uniprot.org/entry/Q9BQ67) (GRWD1_HUMAN)

[uniprot/Q810D6](https://www.uniprot.org/entry/Q810D6) (GRWD1_MOUSE)

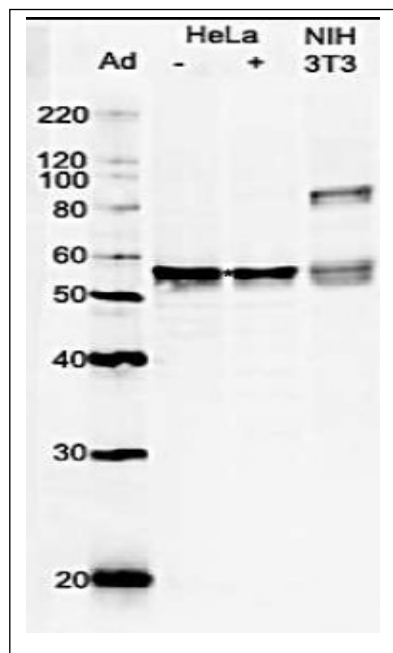


Fig.1. Identification of GRWD1 proteins in whole cell lysates by western blotting with anti-GRWD1 antibody

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 μ 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).

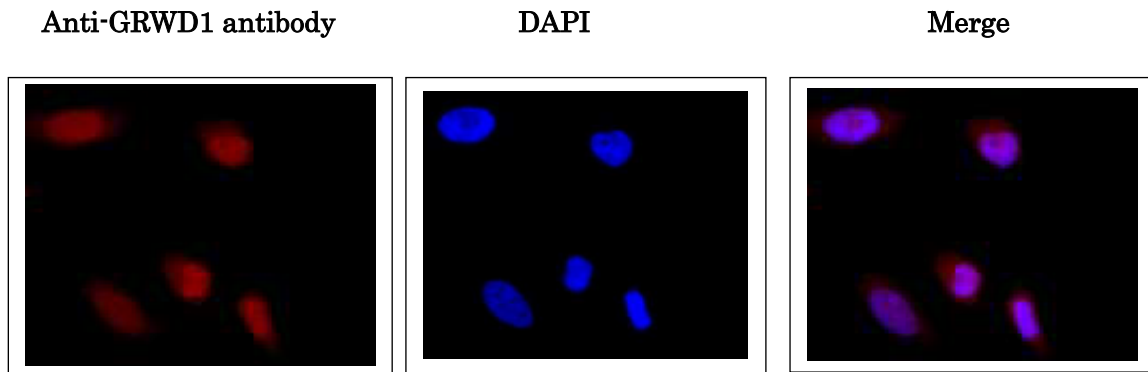


Fig.2. Immunofluorescence staining of GRWD1 protein in HeLa cells.

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.

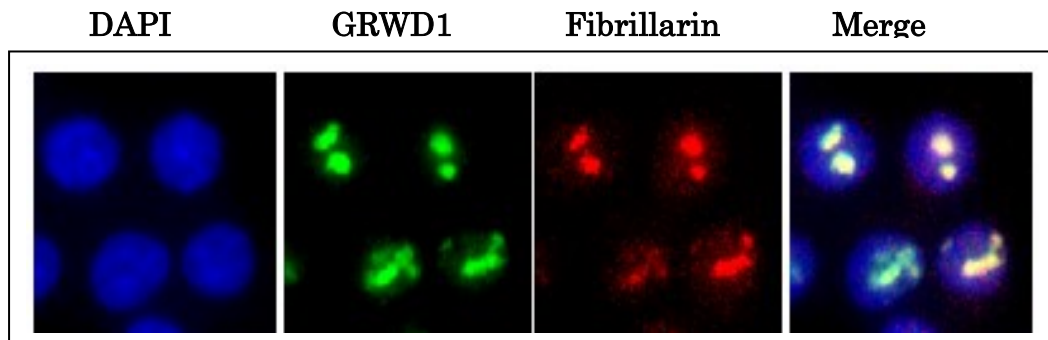


Fig.3. Nucleolar localization of GRWD1.

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)**