Bio Academia
Manufacturer

AS ONE

Anti-GRWD1 antibody, rabbit polyclonal, affinity-purified

70-130 100 µg

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.

Applications
1. Western blotting (1/1,000~1/3,000 dilution)
2. Immunoprecipitation (Assay dependent)
3. Immunofluorescence staining / Immunochemistry (1/100~1/1,000 dilution)

Specification
Immunogen: Purified GST-GRWD1 (human, full-length) expressed in E. coli
Purification: The antiserum was first adsorbed with GST-agarose column and then the pass-through fraction was affiinity-purified with GST-GRWD1 agarose column.
Product: 1 mg/ml in PBS and 50% glycerol. Filter-sterilized. Carrier protein and azide free.
Reactivity: Human, mouse and rat. Other species have not been tested.
Storage: Shipped at 4°C. Upon arrival, spin-down and store at -20°C.

Data Link: uniprot/Q9BQ67 (GRWD1_HUMAN); uniprot/Q810D6 (GRWD1_MOUSE)

Reference: This product has been described and used in the following publication:

1. Sugimoto N. et al. (2008) Identification of novel human Cdt1-binding proteins by a proteomics approach: proteolytic regulation by APC/CCdh1. Mol Biol Cell. 19(3):1007-21.
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)

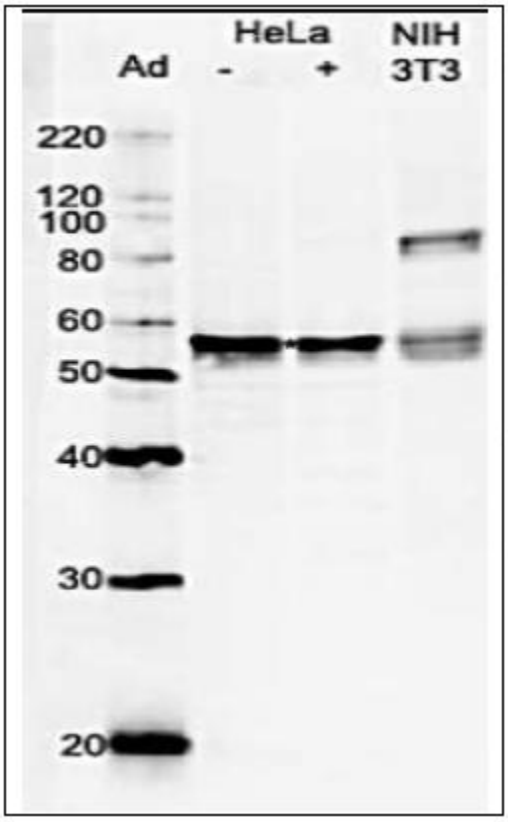

Fig.1. Identication of GRWD1 proteins in whole cell lysates by western blotting with anti-GRWD1 antibody

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 µg. 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).

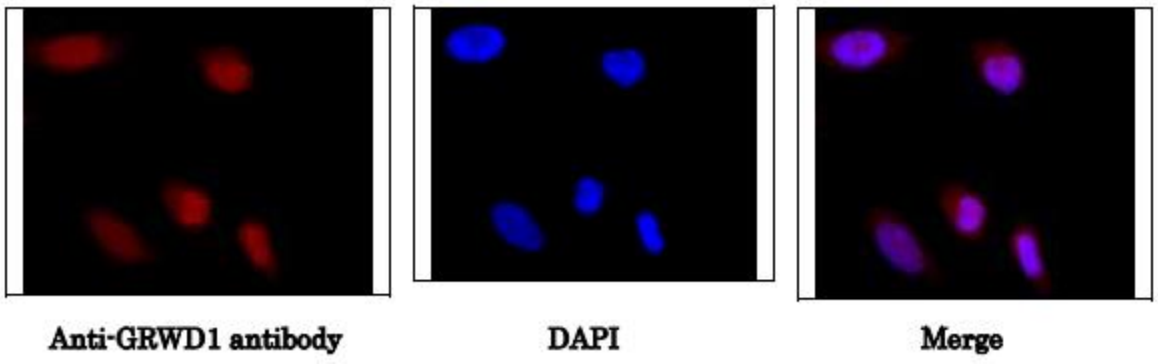

Fig.2. Immunofluorescence staining of GRWD1 protein in HeLa cells

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.

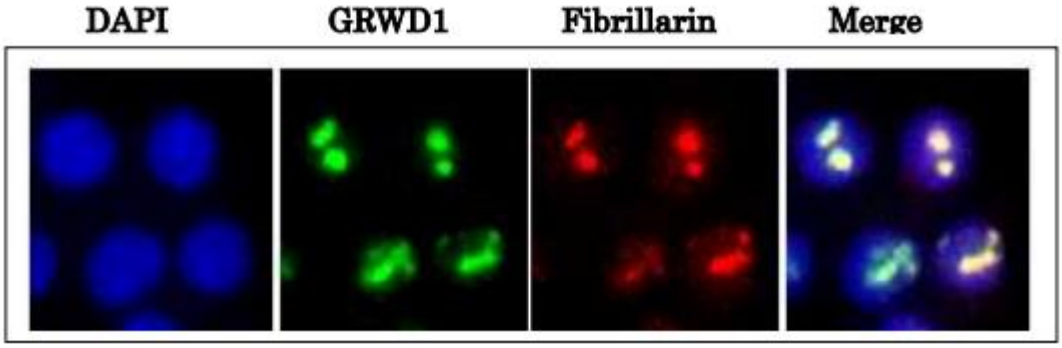

Fig.3. Nucleolar localization of GRWD1. HCT116 cells were first extracted with Triton X-100 to remove nucleoplasmic proteins, double-immunostained with anti-GRWD1 (green) and anti-fibrillarin (red) antibodies as a marker for nucleoli, and counterstained with DAPI.

Key words: WD repeat, KIAA1942, WDR28, METTL18, Phosphorylation