Anti-GWL Antibody [PSH07-69] - BSA and Azide free

HA751181

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Monkey
Applications:	WB, IF-Cell, IP
Molecular Wt:	Predicted band size: 97 kDa
Clone number:	PSH07-69

Description: Serine/threonine kinase that plays a key role in M phase by acting as a regulator of mitosis entry and maintenance. Acts by promoting the inactivation of protein phosphatase 2A (PP2A) during M phase: does not directly inhibit PP2A but acts by mediating phosphorylation and subsequent activation of ARPP19 and ENSA at 'Ser-62' and 'Ser-67', respectively. ARPP19 and ENSA are phosphatase inhibitors that specifically inhibit the PPP2R2D (PR55-delta) subunit of PP2A. Inactivation of PP2A during M phase is essential to keep cyclin-B1-CDK1 activity high. Following DNA damage, it is also involved in checkpoint recovery by being inhibited. Phosphorylates histone protein in vitro; however such activity is unsure in vivo. May be involved in megakaryocyte differentiation.

Immunogen: Recombinant protein within human GWL aa 1-350.

Positive control: HeLa cell lysate, LNCaP cell lysate, HepG2 cell lysate, MDA-MB-231 cell lysate, COS-1 cell lysate, HeLa.

Subcellular location: Cytoplasm, cytoskeleton, microtubule organizing center, centrosome, Nucleus, Cleavage furrow.

Database links: SwissProt: Q96GX5 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

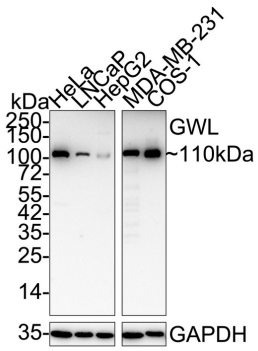

Fig1: Western blot analysis of GWL on different lysates with Rabbit anti-GWL antibody (HA751181) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: LNCaP cell lysate
Lane 3: HepG2 cell lysate
Lane 4: MDA-MB-231 cell lysate
Lane 5: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 97 kDa
Observed band size: 110 kDa

Exposure time: 59 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751181) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

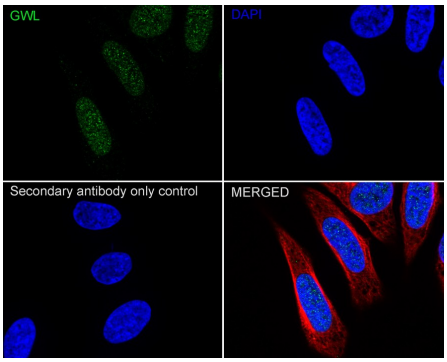

Fig2: Immunocytochemistry analysis of HeLa cells labeling GWL with Rabbit anti-GWL antibody (HA751181) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GWL antibody (HA751181) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/50 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

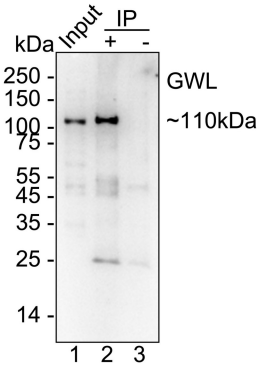

Fig3: GWL was immunoprecipitated from 0.2 mg HeLa cell lysate with HA751181 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751181 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA751181 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA751181 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 40 seconds; ECL: K1802

Background References

1. Gall-Duncan T et al. Antagonistic roles of canonical and Alternative-RPA in disease-associated tandem CAG repeat instability. Cell. 2023 Oct.
2. Yang J et al. Sequential genome-wide CRISPR-Cas9 screens identify genes regulating cell-surface expression of tetraspanins. Cell Rep. 2023 Feb.