Anti-Phospho-Rb (S795) Antibody [PSH06-89] HA722764

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 106 kDa
Clone number:	PSH06-89

Description: The retinoblastoma protein (protein name abbreviated Rb; gene name abbreviated Rb, RB or RB1) is a tumor suppressor protein that is dysfunctional in several major cancers. One function of pRb is to prevent excessive cell growth by inhibiting cell cycle progression until a cell is ready to divide. When the cell is ready to divide, pRb is phosphorylated, inactivating it, and the cell cycle is allowed to progress. It is also a recruiter of several chromatin remodeling enzymes such as methylases and acetylases. pRb belongs to the pocket protein family, whose members have a pocket for the functional binding of other proteins. Should an oncogenic protein, such as those produced by cells infected by high-risk types of human papillomavirus, bind and inactivate pRb, this can lead to cancer. The RB gene may have been responsible for the evolution of multicellularity in several lineages of life including animals.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser795 of human Rb aa 776-825.

Positive control: HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate, HeLa cells treated with 100ng/mL Nocodazole for 18 hours, U-2 OS cell lysate, U-2 OS treated with 50ng/mL Nocodazole for 8 hours cell lysate.

Subcellular location: Nucleus.

Database links: SwissProt: P06400 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:500

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

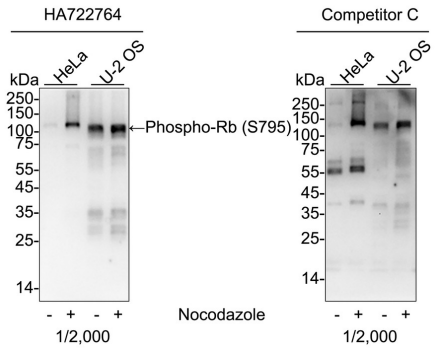

Fig1: Western blot analysis of Phospho-Rb (S795) on different lysates with Rabbit anti-Phospho-Rb (S795) antibody (HA722764) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate
Lane 3: U-2 OS cell lysate
Lane 4: U-2 OS treated with 50ng/mL Nocodazole for 8 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 106 kDa
Observed band size: 106 kDa

Exposure time: Lane 1-4 (left): 40 seconds; Lane 1-4 (right): 1 minute; ECL: K1802;
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 (HA722764) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

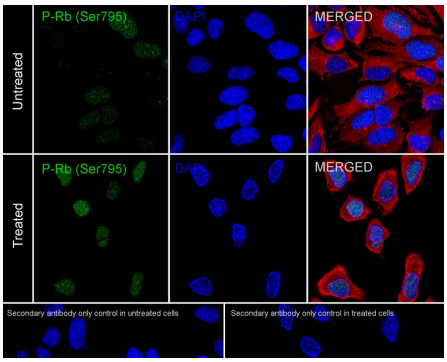

Fig2: Immunocytochemistry analysis of HeLa cells treated with 100ng/mL Nocodazole for 18 hours labeling Phospho-Rb (S795) with Rabbit anti-Phospho-Rb (S795) antibody (HA722764) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-Rb (S795) antibody (HA722764) at 1/500 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 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

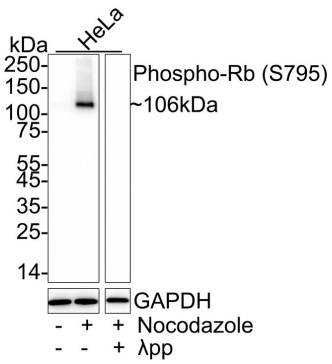

Fig3: Western blot analysis of Phospho-Rb (S795) on different lysates with Rabbit anti-Phospho-Rb (S795) antibody (HA722764) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate
Lane 3: HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 106 kDa
Observed band size: 106 kDa

Exposure time: 3 minutes; 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 (HA722764) 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.

Background References

1. Singh G et al. Tissue-specific response of the RB-E2F1 complex during mammalian hibernation. J Exp Zool A Ecol Integr Physiol. 2022 Dec
2. Ding D et al. Retinoblastoma protein as an intrinsic BRD4 inhibitor modulates small molecule BET inhibitor sensitivity in cancer. Nat Commun. 2022 Oct