Anti-Phospho-Rb (S807/S811) Antibody [PSH11-01] - BSA and Azide free

HA751391

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 106 kDa
Clone number:	PSH11-01

Description: The retinoblastoma protein (protein name abbreviated Rb or pRb; 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 Ser807/Ser811 of Human Rb.

Positive control: K-562 cell lysate, SH-SY5Y cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 100ng/mL Nocodazole for 18 hours cell lysate, human lung tissue, MCF7.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: P06400 Human | P13405 Mouse

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:250
FC	1:1,000
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

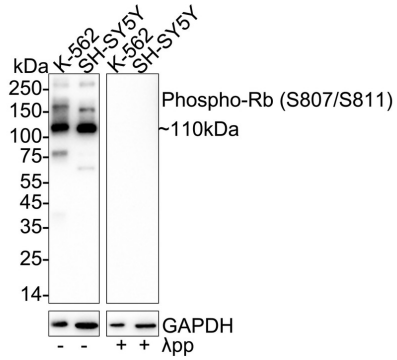

Fig1: Western blot analysis of Phospho-Rb (S807/S811) on different lysates with Rabbit anti-Phospho-Rb (S807/S811) antibody (HA751391) at 1/1,000 dilution.

Lane 1: K-562 cell lysate (no heat) (20 µg/Lane)
Lane 2: SH-SY5Y cell lysate (no heat) (20 µg/Lane)
Lane 3: K-562 cell lysate (no heat), the membrane treated with λpp for 1 hour (20 µg/Lane)
Lane 4: SH-SY5Y cell lysate (no heat), the membrane treated with λpp for 1 hour (20 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 106 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 (HA751391) at 1/1,000 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.

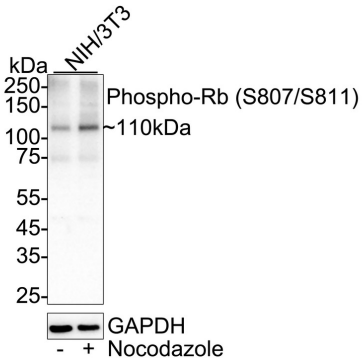

Fig2: Western blot analysis of Phospho-Rb (S807/S811) on different lysates with Rabbit anti-Phospho-Rb (S807/S811) antibody (HA751391) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (no heat) (20 µg/Lane)
Lane 2: NIH/3T3 treated with 100ng/mL Nocodazole for 18 hours cell lysate (no heat) (20 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 106 kDa
Observed band size: 110 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 (HA751391) at 1/1,000 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.

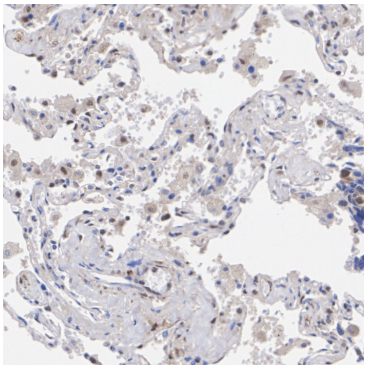

Fig3: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-Phospho-Rb (S807/S811) antibody (HA751391) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751391) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

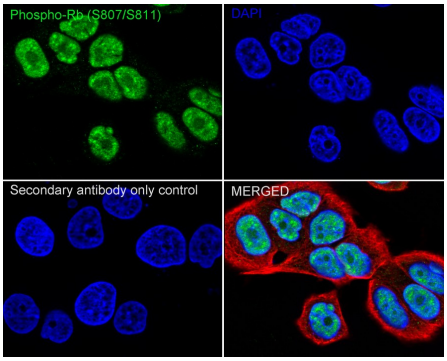

Fig4: Immunocytochemistry analysis of MCF7 cells labeling Phospho-Rb (S807/S811) with Rabbit anti-Phospho-Rb (S807/S811) antibody (HA751391) at 1/250 dilution.

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-Phospho-Rb (S807/S811) antibody (HA751391) at 1/250 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 (HA601187, 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.

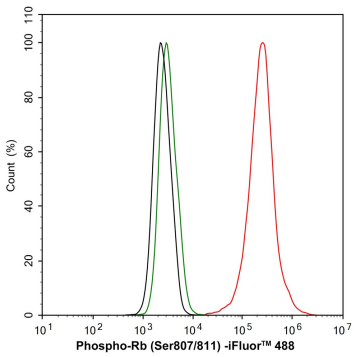

Fig5: Flow cytometric analysis of MCF7 cells labeling Phospho-Rb (S807/S811).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751391, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

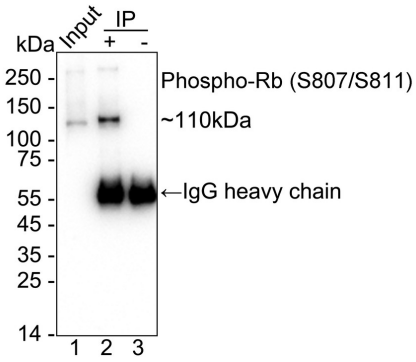

Fig6: Phospho-Rb (S807/S811) was immunoprecipitated from 0.2 mg K-562 cell lysate with HA751391 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751391 at 1/1,000 dilution. Mouse anti Rabbit IgG heavy chain (Fc) secondary antibody (M1003-7) at 1/10,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA751391 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA751391 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

Background References

1. Ni XC et al. Ginsenoside Rb1 inhibits astrocyte activation and promotes transfer of astrocytic mitochondria to neurons against ischemic stroke. Redox Biol. 2022 Aug
2. Qin GW et al. Ginsenoside Rb1 Inhibits Cardiomyocyte Autophagy via PI3K/Akt/mTOR Signaling Pathway and Reduces Myocardial Ischemia/Reperfusion Injury. Am J Chin Med. 2021