Anti-Phospho-Rb (S807) Antibody [SR3-82]

ET1602-36

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 106 kDa
Clone number:	SR3-82

Description: Pediatric cancer retinoblastoma and the formation of other human tumors can be attributed to mutations in the retinoblastoma tumor suppressor gene (Rb). The Rb protein regulates differentiation, apoptosis and cell cycle control by coordinating the cell cycle at G1-S with transcriptional machinery. During G1, cyclin D-dependent kinase-mediated phosphorylation of Rb at Ser 795 marks the conversion of Rb from a transcriptionally repressive, hypophosphorylated state to an inactive, phosphorylated state, which may be sustained through mitosis by differential phosphorylation of up to 16 putative serine or threonine residues, including Ser 249/Thr 252, Thr 373, Thr 356, Ser 780, Ser-807/ Ser 811 and Thr 821/Thr 826. Hypophosphorylated Rb represses the transcription of genes controlling the cell cycle through direct protein-protein interactions and through the recruitment of histone deacetylase.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser807 of Human Rb.

Positive control: K-562 cell lysate, K-562, L929, C6, human tonsil tissue, human lung squamous cell carcinoma tissue, human spleen tissue, mouse spleen tissue, rat spleen tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P06400 Human | P13405 Mouse | P33568 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:100
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

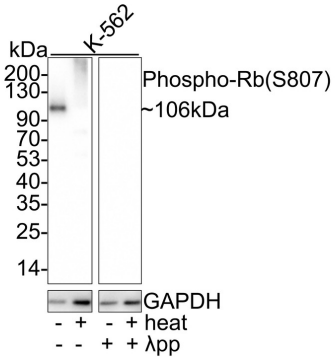

Fig1: Western blot analysis of Phospho-Rb (S807) on different lysates with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/2,000 dilution.

Lane 1: K-562 cell lysate (no heat)
Lane 2: K-562 cell lysate
Lane 3: K-562 cell lysate (no heat), treated with λpp for 1 hour
Lane 4: K-562 cell lysate, treated with λpp for 1 hour

Notice: no heat means the lysate is not boiled.

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 2 minutes;

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 (ET1602-36) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

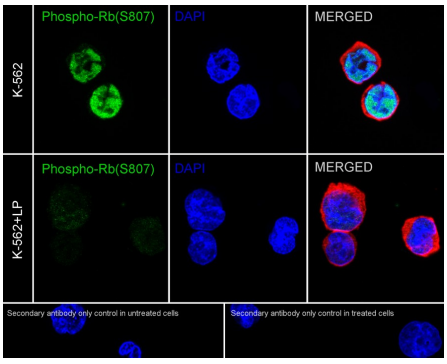

Fig2: Immunocytochemistry analysis of K-562 cells treated with or without λpp labeling Phospho-Rb (S807) with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/100 dilution.

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) antibody (ET1602-36) at 1/100 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.

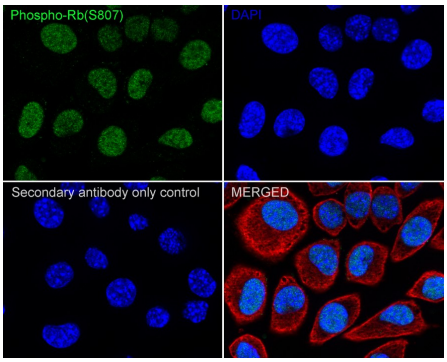

Fig3: Immunocytochemistry analysis of L929 cells labeling Phospho-Rb (S807) with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/100 dilution.

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) antibody (ET1602-36) at 1/100 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.

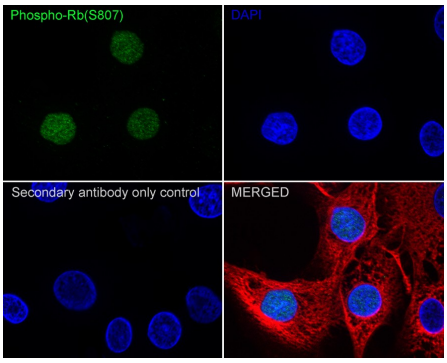

Fig4: Immunocytochemistry analysis of C6 cells labeling Phospho-Rb (S807) with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/50 dilution.

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-Phospho-Rb (S807) antibody (ET1602-36) 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 (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.

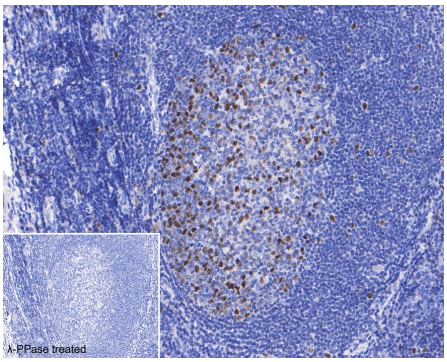

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue untreated / treated with λpp with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/200 dilution.

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 (ET1602-36) at 1/200 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.

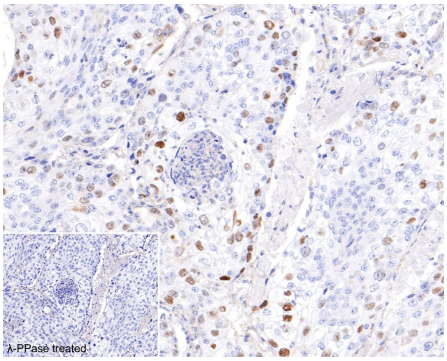

Fig6: Immunohistochemical analysis of paraffin-embedded human lung squamous cell carcinoma tissue untreated / treated with λpp with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/200 dilution.

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 (ET1602-36) at 1/200 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.

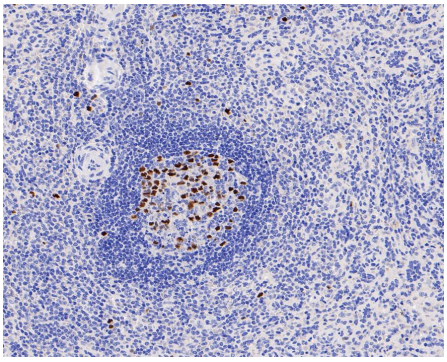

Fig7: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET1602-36) 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.

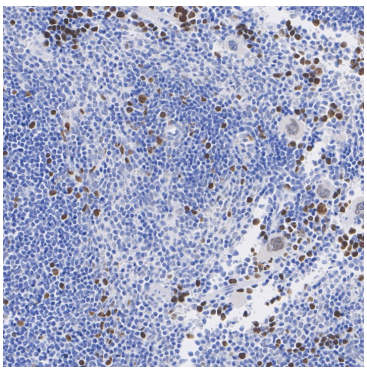

Fig8: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/1,000 dilution.

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 (ET1602-36) 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.

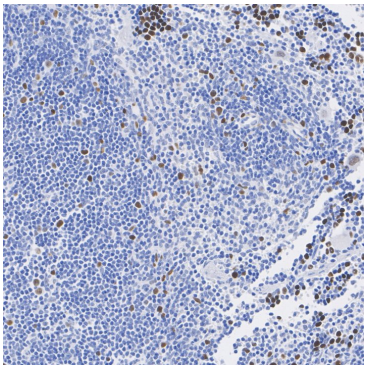

Fig9: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET1602-36) 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.

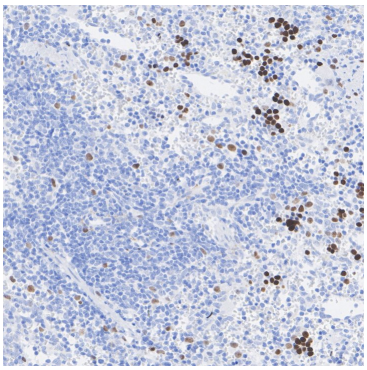

Fig10: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Phospho-Rb (S807) antibody (ET1602-36) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET1602-36) 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.

Background References

1. Tirado-Vélez JM et al. Inhibition of Fatty Acid metabolism reduces human myeloma cells proliferation. PLoS One 7:e46484 (2012).
2. Hyun, TK. et al. 2013. Evaluation of anti-oxidant and anti-cancer properties of Dendropanax morbifera Léveille. Food Chem. 141: 1947-1955.