Anti-PCNA Antibody [A6-G11-R]

HA601172

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IF-Cell, IP
Molecular Wt:	Predicted band size: 29 kDa
Clone number:	A6-G11-R

Description: Auxiliary protein of DNA polymerase delta and is involved in the control of eukaryotic DNA replication by increasing the polymerase's processibility during elongation of the leading strand. Induces a robust stimulatory effect on the 3'-5' exonuclease and 3'-phosphodiesterase, but not apurinic-apyrimidinic (AP) endonuclease, APEX2 activities. Has to be loaded onto DNA in order to be able to stimulate APEX2. Plays a key role in DNA damage response (DDR) by being conveniently positioned at the replication fork to coordinate DNA replication with DNA repair and DNA damage tolerance pathways. Acts as a loading platform to recruit DDR proteins that allow completion of DNA replication after DNA damage and promote postreplication repair: Monoubiquitinated PCNA leads to recruitment of translesion (TLS) polymerases, while 'Lys-63'-linked polyubiquitination of PCNA is involved in error-free pathway and employs recombination mechanisms to synthesize across the lesion.

Immunogen: Recombinant protein within human PCNA aa 1-261.

Positive control: HeLa cell lysate, HEK-293 cell lysate, HCT 116 cell lysate, K-562 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, L6 cell lysate, mouse spleen tissue lysate, rat spleen tissue lysate, HeLa, Raji cell lysate, NIH/3T3 cell lysate, L-929 cell lysate, human liver tissue lysate, HepG2, human lymph node tissue, human melanoma tissue, rat spleen tissue.

Subcellular location: Nucleus

Database links: SwissProt: P12004 Human | P17918 Mouse | P04961 Rat

Recommended Dilutions:

WB	1:20,000-1:50,000
IHC-P	1:2,000
IF-Cell	1:100-1:2,000
IP	1-2μg/sample

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

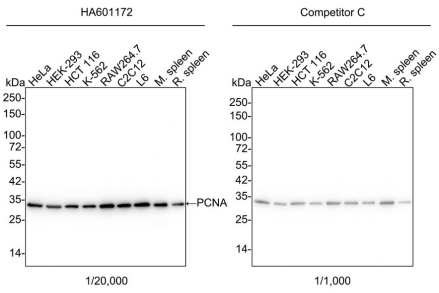

Fig1: Western blot analysis of PCNA on different lysates with Mouse anti-PCNA antibody (HA601172) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HEK-293 cell lysate (15 µg/Lane)
Lane 3: HCT 116 cell lysate (15 µg/Lane)
Lane 4: K-562 cell lysate (15 µg/Lane)
Lane 5: RAW264.7 cell lysate (15 µg/Lane)
Lane 6: C2C12 cell lysate (15 µg/Lane)
Lane 7: L6 cell lysate (15 µg/Lane)
Lane 8: Mouse spleen tissue lysate (15 µg/Lane)
Lane 9: Rat spleen tissue lysate (15 µg/Lane)

Predicted band size: 29 kDa
Observed band size: 34 kDa
Exposure time: 11 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 (HA601172) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

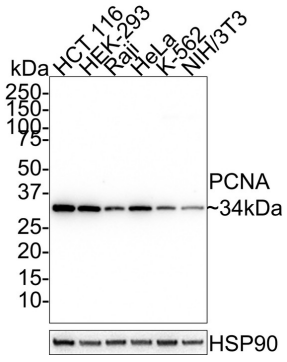

Fig2: Western blot analysis of PCNA on different lysates with Mouse anti-PCNA antibody (HA601172) at 1/20,000 dilution.

Lane 1: HCT 116 cell lysate (20 µg/Lane)
Lane 2: HEK-293 cell lysate (20 µg/Lane)
Lane 3: Raji cell lysate (20 µg/Lane)
Lane 4: HeLa cell lysate (20 µg/Lane)
Lane 5: K-562 cell lysate (20 µg/Lane)
Lane 6: NIH/3T3 cell lysate (20 µg/Lane)

Predicted band size: 29 kDa
Observed band size: 34 kDa
Exposure time: 1 minutes 2 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 (HA601172) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/100,000 dilution was used for 1 hour at room temperature.

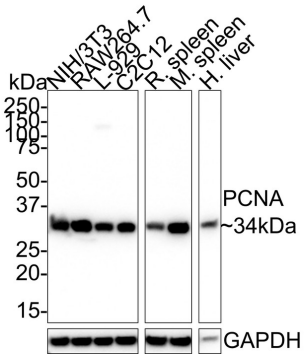

Fig3: Western blot analysis of PCNA on different lysates with Mouse anti-PCNA antibody (HA601172) at 1/20,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: RAW264.7 cell lysate (20 µg/Lane)
Lane 3: L-929 cell lysate (20 µg/Lane)
Lane 4: C2C12 cell lysate (20 µg/Lane)
Lane 5: Rat spleen tissue lysate (40 µg/Lane)
Lane 6: Mouse spleen tissue lysate (40 µg/Lane)
Lane 7: Human liver tissue lysate (40 µg/Lane)

Predicted band size: 29 kDa
Observed band size: 34 kDa

Exposure time: 7 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 (HA601172) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

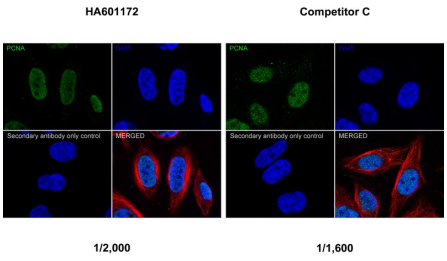

Fig4: Immunocytochemistry analysis of HeLa cells labeling PCNA with Mouse anti-PCNA antibody (HA601172) at 1/2,000 dilution and competitor's antibody at 1/1,600 dilution.

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 Mouse anti-PCNA antibody (HA601172) at 1/2,000 dilution and competitor's antibody at 1/1,600 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

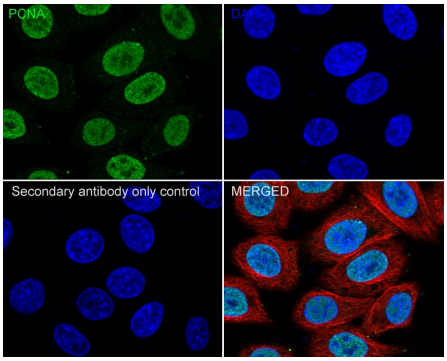

Fig5: Immunocytochemistry analysis of HepG2 cells labeling PCNA with Mouse anti-PCNA antibody (HA601172) at 1/100 dilution.

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 Mouse anti-PCNA antibody (HA601172) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

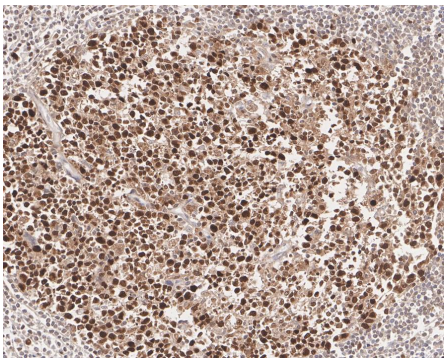

Fig6: Immunohistochemical analysis of paraffin-embedded human lymph node tissue with Mouse anti-PCNA antibody (HA601172) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA601172) at 1/2,000 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.

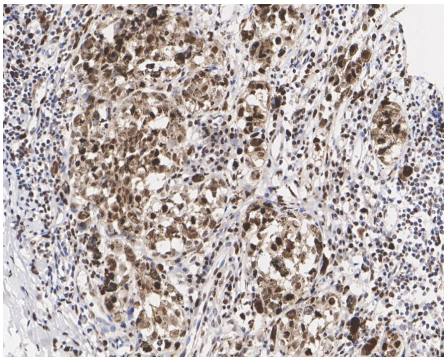

Fig7: Immunohistochemical analysis of paraffin-embedded human melanoma tissue with Mouse anti-PCNA antibody (HA601172) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA601172) at 1/2,000 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.

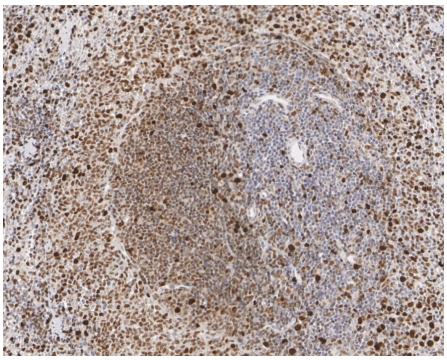

Fig8: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Mouse anti-PCNA antibody (HA601172) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA601172) at 1/2,000 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.

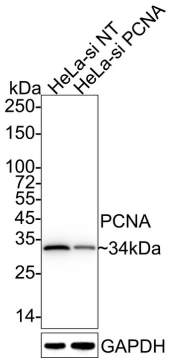

Fig9: Western blot analysis of PCNA on different lysates with Mouse anti-PCNA antibody (HA601172) at 1/20,000 dilution.

Lane 1: HeLa-si NT cell lysate
Lane 2: HeLa-si PCNA cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 29 kDa
Observed band size: 34 kDa

Exposure time: 10 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 (HA601172) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/100,000 dilution was used for 1 hour at room temperature.

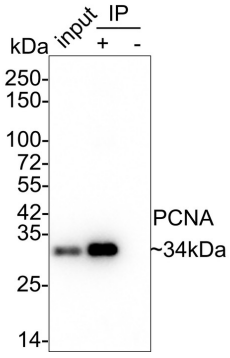

Fig10: PCNA was immunoprecipitated from 0.2 mg HeLa cell lysate with HA601172 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA601172 at 1/10,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA601172 IP in HeLa cell lysate
Lane 3: Mouse IgG instead of HA601172 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 24 seconds; ECL: K1801

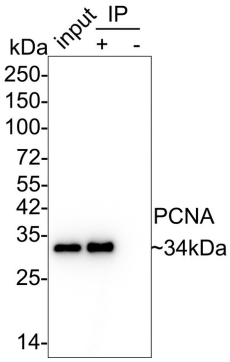

Fig11: PCNA was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA601172 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA601172 at 1/10,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: HA601172 IP in NIH/3T3 cell lysate
Lane 3: Mouse IgG instead of HA601172 in NIH/3T3 cell lysate

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

Background References

1. "Targeting tyrosine phosphorylation of PCNA inhibits prostate cancer growth." Zhao,H., et al. Mol. Cancer Ther. 10: 29-36(2011)
2. "A cancer-associated PCNA expressed in breast cancer has implications as a potential biomarker." Linda H. Malkas, Brittney Shea Herbert, Waleed Abdel-Aziz, Lacey E. Dobrolecki, et al. Proc Natl Acad Sci. 103(51)(2006)