Anti-Cyclin T1 Antibody [PSH09-66] - BSA and Azide free

HA751308

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IP, ChIP
Molecular Wt:	Predicted band size: 81 kDa
Clone number:	PSH09-66

Description: Cyclin-T1 is a protein that in humans is encoded by the CCNT1 gene. The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns that contribute to the temporal coordination of each mitotic event. This cyclin tightly associates with CDK9 kinase, and was found to be a major subunit of the transcription elongation factor p-TEFb. The kinase complex containing this cyclin and the elongation factor can interact with, and act as a cofactor of human immunodeficiency virus type 1 (HIV-1) Tat protein, and was shown to be both necessary and sufficient for full activation of viral transcription. This cyclin and its kinase partner were also found to be involved in the phosphorylation and regulation of the carboxy-terminal domain (CTD) of the largest RNA polymerase II subunit.

Immunogen: Recombinant protein within human Cyclin T1 aa 251-530.

Positive control: Jurkat cell lysate, K-562 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, NIH/3T3 cell lysate, C6 cell lysate, mouse liver tissue, rat liver tissue, NIH/3T3, C6.

Subcellular location: Nucleus.

Database links: SwissProt: O60563 Human | Q9QWV9 Mouse
Entrez Gene: 315291 Rat

Recommended Dilutions:

WB	1:2,000
IHC-P	1:2,000
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample
ChIP	Use 2~5 μg for 25 μg of chromatin.

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

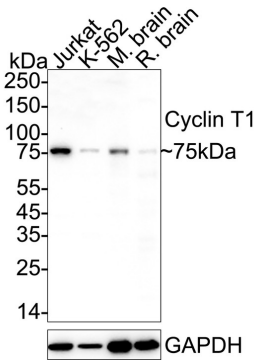

Fig1: Western blot analysis of Cyclin T1 on different lysates with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/2,000 dilution.

Lane 1: Jurkat cell lysate (20 µg/Lane)
Lane 2: K-562 cell lysate (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (40 µg/Lane)
Lane 4: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 81 kDa
Observed band size: 75 kDa

Exposure time: 25 seconds; 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 (HA751308) 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.

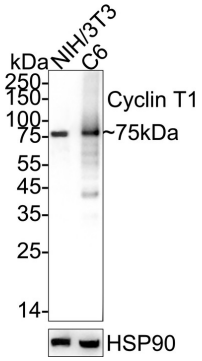

Fig2: Western blot analysis of Cyclin T1 on different lysates with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/2,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 81 kDa
Observed band size: 75 kDa

Exposure time: 1 minute; 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 (HA751308) 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.

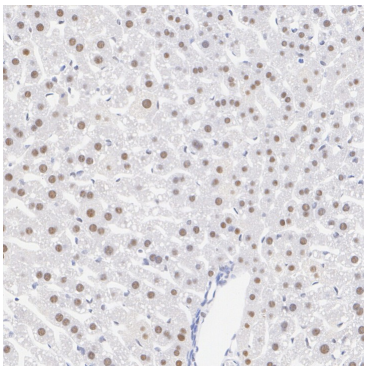

Fig3: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/2,000 dilution.

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 (HA751308) 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.

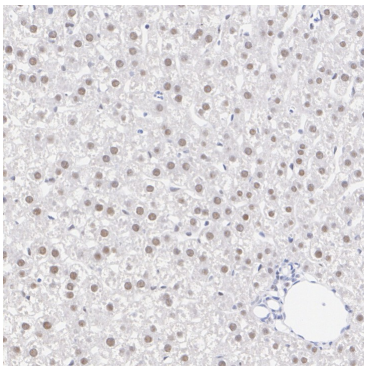

Fig4: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/2,000 dilution.

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 (HA751308) 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.

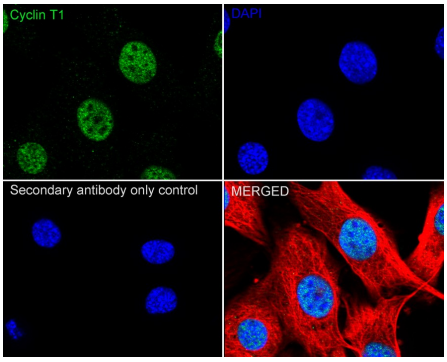

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling Cyclin T1 with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/100 dilution.

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-Cyclin T1 antibody (HA751308) 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.

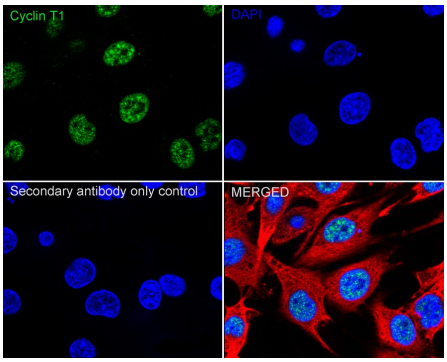

Fig6: Immunocytochemistry analysis of C6 cells labeling Cyclin T1 with Rabbit anti-Cyclin T1 antibody (HA751308) at 1/100 dilution.

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-Cyclin T1 antibody (HA751308) 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.

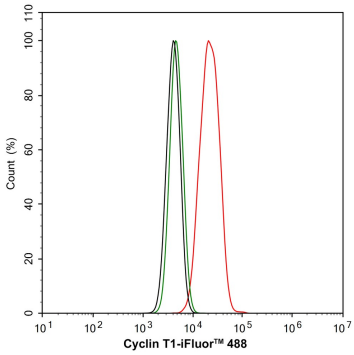

Fig7: Flow cytometric analysis of NIH/3T3 cells labeling Cyclin T1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751308, 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).

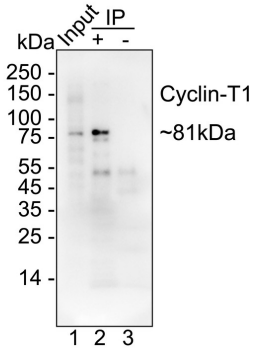

Fig8: Cyclin T1 was immunoprecipitated from 0.2 mg Jurkat cell lysate with HA751308 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751308 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: HA751308 IP in Jurkat cell lysate
Lane 3: Rabbit IgG instead of HA751308 in Jurkat cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 37 seconds; ECL: K1802

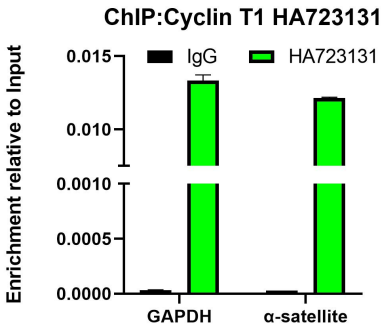

Fig9: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Cyclin T1 (HA751308) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Lin R et al. Discovery of HyT-Based Degraders of CDK9-Cyclin T1 Complex. Chem Biodivers. 2023 Aug
2. Cheng SS et al. Inhibition of the CDK9-cyclin T1 protein-protein interaction as a new approach against triple-negative breast cancer. Acta Pharm Sin B. 2022 Mar