Anti-Phospho-CDK1 (T161) Antibody [PSH03-42] HA721987

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	PSH03-42

Description: Cyclin-dependent kinase 1 also known as CDK1 or cell division cycle protein 2 homolog is a highly conserved protein that functions as a serine/threonine protein kinase, and is a key player in cell cycle regulation. It has been highly studied in the budding yeast S. cerevisiae, and the fission yeast S. pombe, where it is encoded by genes cdc28 and cdc2, respectively. With its cyclin partners, Cdk1 forms complexes that phosphorylate a variety of target substrates (over 75 have been identified in budding yeast); phosphorylation of these proteins leads to cell cycle progression.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Thr161 of CDK1.

Positive control: HeLa cell lysate, HeLa treated with 100nM Calyculin A for 30 minutes cell lysate, Jurkat cell lysate, Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, PC-12 cell lysate, PC-12 treated with UV for 1 hour cell lysate, human cervix cancer tissue, Jurkat cells treated with 100nM Calyculin A for 30 minutes, HeLa cells treated with 100nM Calyculin A for 30 minutes, NIH/3T3 cells treated with 100nM Calyculin A for 30 minutes.

Subcellular location: Nucleus, Mitochondrion, Cytoplasm, cytoskeleton, microtubule organizing center, spindle.

Database links: SwissProt: P06493 Human | P11440 Mouse | P39951 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:2,500
IHC-P	1:200
FC	1:100

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

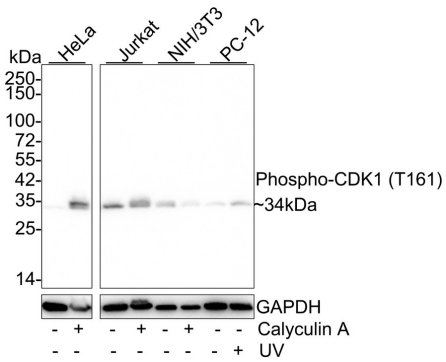

Fig1: Western blot analysis of Phospho-CDK1 (T161) on different lysates with Rabbit anti-Phospho-CDK1 (T161) antibody (HA721987) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 3: Jurkat cell lysate
Lane 4: Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 7: PC-12 cell lysate
Lane 8: PC-12 treated with UV for 1 hour cell lysate

Lysates/proteins at 30 µg/Lane.

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

Exposure time: 5 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 (HA721987) 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.

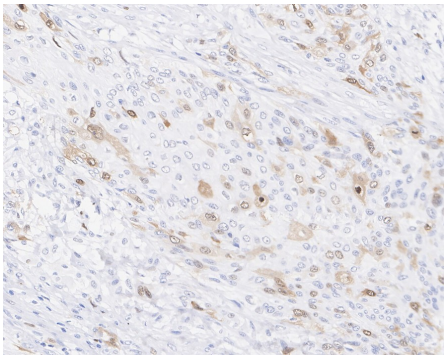

Fig2: Immunohistochemical analysis of paraffin-embedded human cervix cancer tissue with Rabbit anti-Phospho-CDK1 (T161) antibody (HA721987) at 1/200 dilution.

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 (HA721987) 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.

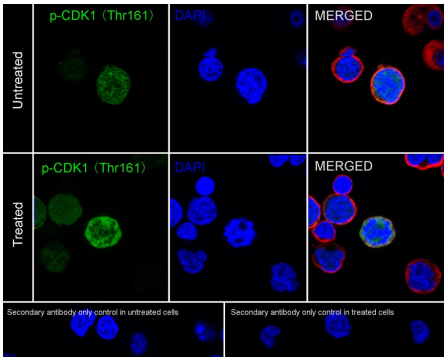

Fig3: Immunocytochemistry analysis of Jurkat cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-CDK1 (T161) with Rabbit anti-Phospho-CDK1 (T161) antibody (HA721987) at 1/2,500 dilution.

Cells were fixed in 100% precooled methanol 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-CDK1 (T161) antibody (HA721987) at 1/2,500 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.

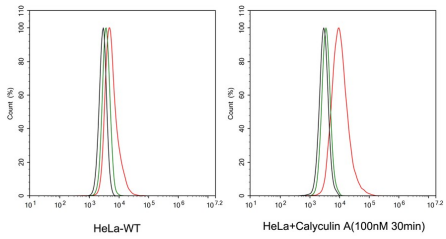

Fig4: Flow cytometric analysis of HeLa cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-CDK1 (T161).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721987, 1/100) (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).

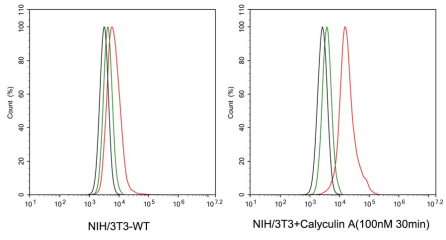

Fig5: Flow cytometric analysis of NIH/3T3 cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-CDK1 (T161).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721987, 1/100) (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).

Background References

1. Haneke K et al. CDK1 couples proliferation with protein synthesis. J Cell Biol. 2020 Mar
2. Michowski W et al. Cdk1 Controls Global Epigenetic Landscape in Embryonic Stem Cells. Mol Cell. 2020 May