Anti-Phospho-Chk1 (S345) Antibody [PS01-17] - BSA and Azide free

HA750523

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	PS01-17

Description: Checkpoint kinase 1, commonly referred to as Chk1, is a serine/threonine-specific protein kinase that, in humans, is encoded by the CHEK1 gene. Chk1 coordinates the DNA damage response (DDR) and cell cycle checkpoint response. Activation of Chk1 results in the initiation of cell cycle checkpoints, cell cycle arrest, DNA repair and cell death to prevent damaged cells from progressing through the cell cycle. Checkpoint kinases (Chks) are protein kinases that are involved in cell cycle control. Two checkpoint kinase subtypes have been identified, Chk1 and Chk2. Chk1 is a central component of genome surveillance pathways and is a key regulator of the cell cycle and cell survival. Chk1 is required for the initiation of DNA damage checkpoints and has recently been shown to play a role in the normal (unperturbed) cell cycle. Chk1 impacts various stages of the cell cycle including the S phase, G2/M transition and M phase. In addition to mediating cell cycle checkpoints, Chk1 also contributes to DNA repair processes, gene transcription, egg production, embryo development, cellular responses to HIV infection and somatic cell viability.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser345 of human Chk1.

Positive control: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate, NIH/3T3 treated with UV for 20 minutes then recover for 2 hours cell lysate, HeLa treated with Etoposide cell lysate, HeLa treated with Hydroxyurea cell lysate, HeLa treated with 4mM Hydroxyurea for 20 hours.

Subcellular location: Nucleus, Chromosome, Cytoplasm, cytoskeleton, microtubule organizing center, centrosome.

Database links: SwissProt: O14757 Human | O35280 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:200

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

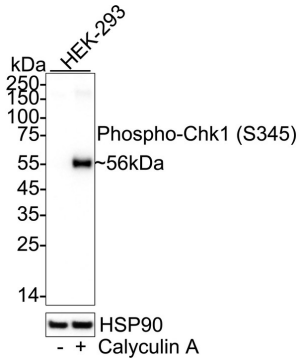

Fig1: Western blot analysis of Phospho-Chk1 (S345) on different lysates with Rabbit anti-Phospho-Chk1 (S345) antibody (HA750523) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 56 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 (HA750523) at 1/1,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

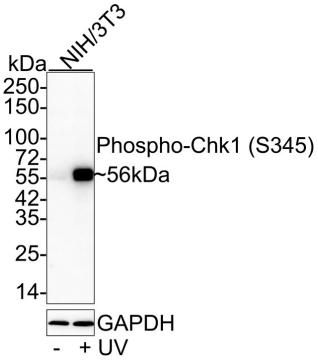

Fig2: Western blot analysis of Phospho-Chk1 (S345) on different lysates with Rabbit anti-Phospho-Chk1 (S345) antibody (HA750523) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with UV for 20 minutes then recover for 2 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 56 kDa

Exposure time: 1 minute;

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 (HA750523) 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.

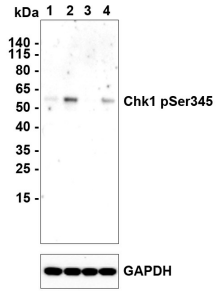

Fig3: Western blot analysis of Phospho-Chk1 (S345) on different lysates with Rabbit anti-Phospho-Chk1 (S345) antibody (HA750523) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with Etoposide cell lysate
Lane 3: HeLa cell lysate
Lane 4: HeLa treated with Hydroxyurea cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 56 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 (HA750523) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

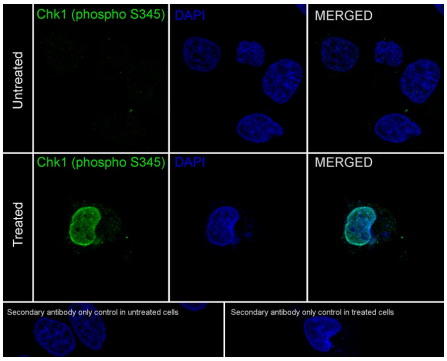

Fig4: Immunocytochemistry analysis of HeLa cells treated with or without 4mM Hydroxyurea for 20 hours labeling Phospho-Chk1 (S345) with Rabbit anti-Phospho-Chk1 (S345) antibody (HA750523) at 1/200 dilution. Image shown an increased nuclear staining upon Hydroxyurea treatment.

Cells were fixed in 4% paraformaldehyde for 20 minutes at 37 ℃, permeabilized with 0.1% Triton X-100 in PBS permeabilization for 5 minutes, and then blocked with 2% negative goat serum for 60 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-Chk1 (S345) antibody (HA750523) at 1/200 dilution in 1% BSA 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.

Background References

1. Klomp JE et al. CHK1 protects oncogenic KRAS-expressing cells from DNA damage and is a target for pancreatic cancer treatment. Cell Rep. 2021 Nov
2. Wen Y et al. EZH2 activates CHK1 signaling to promote ovarian cancer chemoresistance by maintaining the properties of cancer stem cells. Theranostics. 2021 Jan