Anti-Phospho-Chk1 (S296) Antibody [SN06-50]

ET1611-76

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	SN06-50

Description: Cell cycle events are regulated by the sequential activation and deactivation of cyclin dependent kinases (Cdks) and by proteolysis of cyclins. Chk1 and Chk2 are involved in these processes as regulators of Cdks. Chk1 and Chk2 both function as essential components in the G2 DNA damage checkpoint by phosphorylating Cdc25C in response to DNA damage. Phosphorylation inhibits Cdc25C activity, thereby blocking mitosis. Cdc25A, Cdc25B and Cdc25C protein tyrosine phosphatases function as mitotic activators by dephosphorylating Cdc2 p34 on regulatory tyrosine residues. It has also been shown that Chk1 can phosphorylate Wee1 in vitro, providing evidence that the hyperphosphorylated form of Wee1, seen in cells delayed by Chk1 overexpression, is due to phosphorylation by Chk1.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser296 of human Chk1

Positive control: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate, human tonsil tissue, human breast cancer tissue, HEK-293 cells treated with 100nM Calyculin A for 30 minutes.

Subcellular location: Chromosome, Cytoplasm, Cytoskeleton, Nucleus.

Database links: SwissProt: O14757 Human | O35280 Mouse | Q91ZN7 Rat

Recommended Dilutions:

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

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

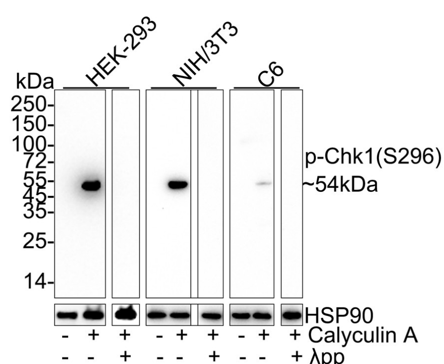

Fig1: Western blot analysis of Phospho-Chk1 (S296) on different lysates with Rabbit anti-Phospho-Chk1 (S296) antibody (ET1611-76) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate
Lane 3: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate, then the membrane treated with λpp for 1 hour
Lane 4: NIH/3T3 cell lysate
Lane 5: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 6: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour
Lane 7: C6 cell lysate
Lane 8: C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate
Lane 9: C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

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

Exposure time: Lane 1-3: 20 seconds; Lane 4-9: 3 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 (ET1611-76) 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.

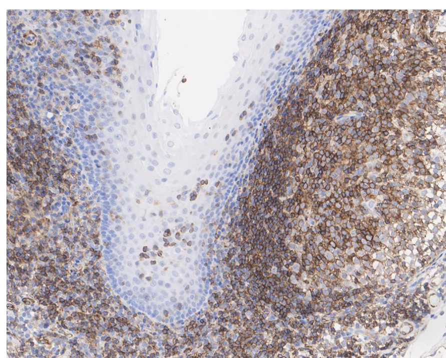

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Phospho-Chk1 (S296) antibody (ET1611-76) at 1/1,000 dilution.

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 (ET1611-76) 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.

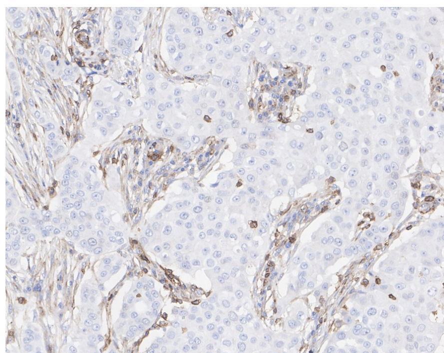

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-Chk1 (S296) antibody (ET1611-76) at 1/200 dilution.

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 (ET1611-76) 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.

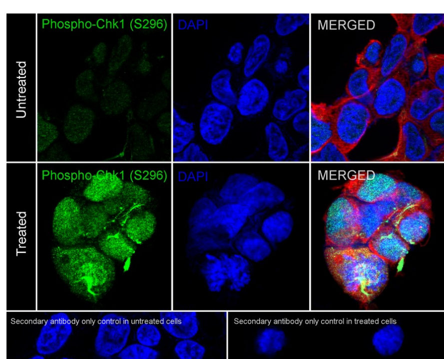

Fig4: Immunocytochemistry analysis of HEK-293 cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-Chk1 (S296) with Rabbit anti-Phospho-Chk1 (S296) antibody (ET1611-76) at 1/100 dilution.

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 Rabbit anti-Phospho-Chk1 (S296) antibody (ET1611-76) 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 (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.

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Bryant C et al. Inhibition of the checkpoint kinase Chk1 induces DNA damage and cell death in human Leukemia and Lymphoma cells. Mol Cancer 13:147 (2014).
2. Okita N et al. CHK1 cleavage in programmed cell death is intricately regulated by both caspase and non-caspase family proteases. Biochim Biophys Acta 1830:2204-13 (2013).