Anti-Chk2 Antibody [SC604]

ET1610-52

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 61 kDa
Clone number:	SC604

Description: Serine/threonine-protein kinase which is required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of DNA double-strand breaks. May also negatively regulate cell cycle progression during unperturbed cell cycles. Following activation, phosphorylates numerous effectors preferentially at the consensus sequence [L-X-R-X-X-S/T]. Regulates cell cycle checkpoint arrest through phosphorylation of CDC25A, CDC25B and CDC25C, inhibiting their activity. Inhibition of CDC25 phosphatase activity leads to increased inhibitory tyrosine phosphorylation of CDK-cyclin complexes and blocks cell cycle progression. May also phosphorylate NEK6 which is involved in G2/M cell cycle arrest. Regulates DNA repair through phosphorylation of BRCA2, enhancing the association of RAD51 with chromatin which promotes DNA repair by homologous recombination. Also stimulates the transcription of genes involved in DNA repair (including BRCA2) through the phosphorylation and activation of the transcription factor FOXM1. Regulates apoptosis through the phosphorylation of p53/TP53, MDM4 and PML. Phosphorylation of p53/TP53 at 'Ser-20' by CHEK2 may alleviate inhibition by MDM2, leading to accumulation of active p53/TP53. Phosphorylation of MDM4 may also reduce degradation of p53/TP53. Also controls the transcription of pro-apoptotic genes through phosphorylation of the transcription factor E2F1. Tumor suppressor, it may also have a DNA damage-independent function in mitotic spindle assembly by phosphorylating BRCA1. Its absence may be a cause of the chromosomal instability observed in some cancer cells. Promotes the CCAR2-SIRT1 association and is required for CCAR2-mediated SIRT1 inhibition.

Immunogen:	Recombinant protein within Human Chk2 aa 10-200 / 543.
Positive control:	A549 cell lysate, HeLa, human testis tissue.
Subcellular location:	Nucleus.
Database links:	SwissProt: O96017 Human
Recommended Dilutions:	
WB	1:1,000-1:2,000
IF-Cell	1:50
IF-Tissue	1:50-1:200
IHC-P	1:1,000
IP	1-2μg/sample
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

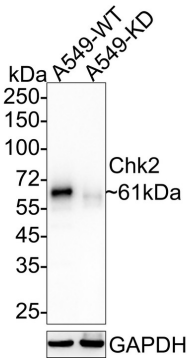

Fig1: Western blot analysis of Chk2 on different lysates with Rabbit anti-Chk2 antibody (ET1610-52) at 1/1,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si Chk2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 61 kDa
Observed band size: 61 kDa

Exposure time: 40 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 (ET1610-52) 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.

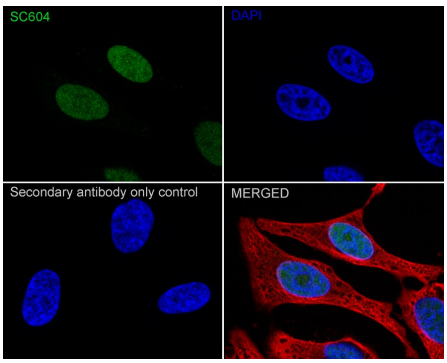

Fig2: Immunocytochemistry analysis of HeLa cells labeling Chk2 with Rabbit anti-Chk2 antibody (ET1610-52) at 1/50 dilution.

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-Chk2 antibody (ET1610-52) at 1/50 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.

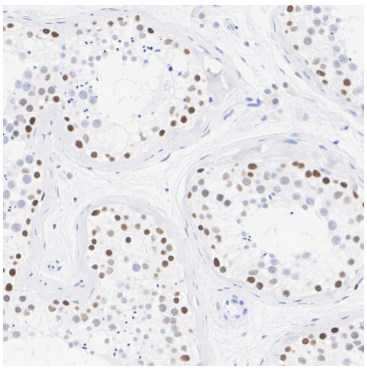

Fig3: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Chk2 antibody (ET1610-52) at 1/1,000 dilution.

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 (ET1610-52) 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.

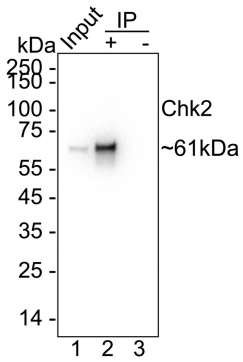

Fig4: Chk2 was immunoprecipitated from 0.2 mg A549 cell lysate with ET1610-52 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1610-52 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 cell lysate (input)
Lane 2: ET1610-52 IP in A549 cell lysate
Lane 3: Rabbit IgG instead of ET1610-52 in A549 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 10 seconds; ECL: K1801

Background References

1. Li WF et al. WISP-1 contributes to fractionated irradiation-induced radioresistance in esophageal carcinoma cell lines and mice. PLoS One 9:e94751 (2014).
2. Sowd GA et al. SV40 utilizes ATM kinase activity to prevent non-homologous end joining of broken viral DNA replication products. PLoS Pathog 10:e1004536 (2014).