

Certificate of Analysis**Product:** Affinity Purified Anti-Human CHK2 pT68 [Rabbit]**Code:** 600-401-280**Lot #:** 15804**Size:** 100 µg**Physical State:** Liquid (sterile filtered)**Antibody Concentration:** 1.0 mg/ml (by UV absorbance at 280 nm)**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2**Stabilizer:** None**Preservative:** 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: CHK2 (also known as CHEK2, Protein kinase CHK2 isoform α , and checkpoint-like protein) is a serine/threonine-protein kinase involved in the control of cell cycle checkpoints and may also participate in transduction of the DNA damage and replicational stress signals. CHK2 is the mammalian ortholog of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases. The amino-terminal domain of CHK2 contains a series of seven serine and threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases. Indeed, after DNA damage by ionizing radiation (IR), UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR. The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to autophosphorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain. CHK2 inhibits CDC25C phosphatase by phosphorylating it on Ser-216, preventing the entry into mitosis. This kinase may have a role in meiosis as well. Kinase activity is up regulated by autophosphorylation and the protein is rapidly phosphorylated in response to DNA damage and to replication block.

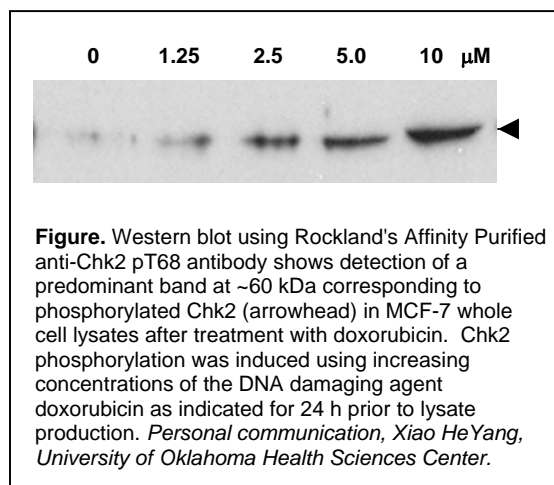
CHK2 shows a nuclear localization and is highly expressed in testis, spleen, colon and peripheral blood leukocytes. Low levels of expression are found in other tissues. Defects in CHEK2 are associated with Li-Fraumeni syndrome (LFS); a highly penetrant familial cancer phenotype usually associated with inherited mutations in p53/TP53. Defects in CHEK2 are also found in some patients with prostate cancer (CaP) or osteosarcoma (OSRC). Substantial amino acid variants exist in some cancer tissues. Numerous truncated splice variants exist for this protein.

Application Note(s): This affinity purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 61 kDa in size corresponding to CHK2 by western blotting in the appropriate cell lysate or extract. Less than 1% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pT68 of CHK2.

Recommended Dilutions:

ELISA
WESTERN BLOT
IF MICROSCOPY
OTHER APPLICATIONS

1:10,000 - 1:50,000
 1:200 - 1:2,000
 User Optimized
 User Optimized



Purity and Specificity: This affinity purified antibody is directed against the phosphorylated form of human CHK2 at the pT68 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human CHK2 pT68 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human CHK2 is minimal by ELISA. The antibody does not cross-react with Chk2 phosphorylated at other sites. A BLAST analysis was used to suggest reactivity with this protein from human and chimpanzee based on 100% homology for the immunogen sequence. Cross reactivity with CHK2 protein from mouse and rat may occur as sequence homology varies by one amino acid residues in this sequence (90% homology). Cross reactivity with CHK2 homologues from other sources has not been determined.

Relevant Link(s): Swiss Prot: [O96017](#) NCBI Link [O96017](#)

Protein Sequence: Human CHK2, 543 aa, predicted MW 60.9 kDa

1	msresdveaq	qshgssacsq	phgsvtqsqg	sssqsqgiss	sststmpnss	qsshsssgtl
61	ssletvstqe	lysipedqep	edqepeeptp	apwarlwalq	dgfanelcvn	dnywfgdrks
121	ceycfdepll	krtdkyrtys	kkhfrifrev	gpknsyiayi	edhsgngtfv	ntelvgkgkr
181	rplnnnseia	lslsrnkfvf	ffdltdvdds	vypkaldrey	imsktlgsga	cgevklafer
241	ktckkvaiki	iskrkfaigs	areadpalnv	eteieilkkl	nhpciikikn	ffdaedyyiv
301	lelmegegelf	dkvvgnkrk	eatcklyfyq	mllavqylhe	ngiihrdlkp	envllssqee
361	dcliokitdfg	hskilgetsl	mrtlctgpty	lapevlsvg	tagynravdc	wslgvilfic
421	lsgypfseh	rtqvsldqj	tsgkynfipe	vvaevsekal	dlvkkllvvd	pkarfttea
481	lrhplwqded	mkrkfqdlls	eenestalp	vlaqpstrk	rpregeaega	ettkrpavca
541	avl					

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding aa 64-73 of Human CHK2.

General References:

Blasina, A., de Weyer, I.V., Laus, M.C., Luyten, W.H., Parker, A.E. and McGowan, C.H. (1999) A human homologue of the checkpoint kinase Cds1 directly inhibits Cdc25 phosphatase. *Curr. Biol.* **9** (1), 1-10.

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#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	MaxTag TM Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

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