OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

R1536 Polyclonal Antibody to CHK2 pThr68 - Purified

Alternate names: CHEK-2, CHEK2, CHK-2, CHK2 checkpoint homolog, Cds1, RAD53, Serine/threonine-protein kinase Chk2

Quantity: 0.1 mg

Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Background: CHK2 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.

Uniprot ID: O96017

NCBI: NP_001005735.1

GeneID: 11200

Host: Rabbit

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.

AA Sequence:

```
Human CHK2, 543 aa, predicted MW 60.9 kDa
1 msresdveaq qshgssacsq phgsvtqsqg sssqsqgiss sststmpnss qsshsssgtl
61 ssletvstqe lysipedqep edqepeeptp apwarlwalq dgfanlecvn dnywfgrdks
121 ceycfdepll krtdkyrtys kkhfrifrev gpknsyiayi edhsgngtfv ntelvgkgkr
181 rplnnnseia lslsrnkvfv ffdltvddqs vypkalrdey imsktlgsga cgevklafer
241 ktckkvaiki iskrkfaigs areadpalnv eteieilkkl nhpciikikn ffdaedyyiv
301 lelmeggelf dkvvgnkrlk eatcklyfyq mllavqylhe ngiihrdlkp envllssqee
361 dclikitdfg hskilgetsl mrtlcgtpty lapevlvsvg tagynravdc wslgvilfic
421 lsgyppfseh rtqvslkdqi tsgkynfipe vwaevsekal dlvkkllvvd pkarfttеea
481 lrhpwlqded mkrkfqdlls eenestalpq vlaqpstsrk rpregeaega ettkrpavca
541 avl
```

Format:

State: Liquid (sterile filtered) purified Ig fraction.
Purification: Immunoaffinity chromatography.
Buffer System: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, containing 0.01% Sodium Azide as preservative.

Applications:

Suitable for use in ELISA (1/10,000-1/50,000) and Western blot (1/200-1/2,000). Expect a band ~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.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

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.

Storage:

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.
Shelf life: one year from despatch.

General Readings:

1. Blasina A, de Weyer IV, Laus MC, Luyten WH, Parker AE, McGowan CH. A human homologue of the checkpoint kinase Cds1 directly inhibits Cdc25 phosphatase. Curr Biol. 1999 Jan 14;9(1):1-10. PubMed PMID: 9889122.
2. Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998 Dec 4;282(5395):1893-7. PubMed PMID: 9836640.
3. Brown AL, Lee CH, Schwarz JK, Mitiku N, Piwnica-Worms H, Chung JH. A human Cds1-related kinase that functions downstream of ATM protein in the cellular response to DNA damage. Proc Natl Acad Sci U S A. 1999 Mar 30;96(7):3745-50. PubMed PMID: 10097108.
4. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science. 1999 Dec 24;286(5449):2528-31. PubMed PMID: 10617473.
5. Allinen M, Huusko P, Mäntyniemi S, Launonen V, Winqvist R. Mutation analysis of the CHK2 gene in families with hereditary breast cancer. Br J Cancer. 2001 Jul 20;85(2):209-12. PubMed PMID: 11461078.
6. Miller CW, Ikezoe T, Krug U, Hofmann WK, Tavor S, Vegesna V, et al. Mutations of the CHK2 gene are found in some osteosarcomas, but are rare in breast, lung, and ovarian tumors. Genes Chromosomes Cancer. 2002 Jan;33(1):17-21. PubMed PMID: 11746983.
7. Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, et al. Mutations in CHEK2 associated with prostate cancer risk. Am J Hum Genet. 2003 Feb;72(2):270-80. Epub 2003 Jan 17. PubMed PMID: 12533788.

Pictures:

Western blot using Affinity Purified anti-Chk2 pT68 antibody shows detection of a predominant band at ~60 kDa corresponding to phosphorylated Chk2 (arrowhead) in MCF-7 whole cell lysates after treatment with doxorubicin. Chk2 phosphorylation was induced using increasing concentrations of the DNA damaging agent doxorubicin as indicated for 24 h prior to lysate production. Personal communication, Xiao HeYang, University of Oklahoma Health Sciences Center.