CHK2 [GST-tagged]

Kinase

Alternate Names: Serine/threonine-protein kinase Chk2, Checkpoint kinase 2, CHEK2, RAD53

Cat. No.	**66-0019-050**	Quantity:	50 µg
Lot. No.	**30298**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. The checkpoint kinases CHK1 and CHK2 are serine/threonine protein kinases that are key components of the intracellular signalling network which responds to damaged DNA, and co-ordinates activation of cell cycle checkpoints with initiation and implementation of DNA repair. Although similarly named, CHK1 and CHK2 are structurally distinct and fulfil different roles in the signalling network Other kinases involved in cell cycle checkpoints are WEE1, p38 and MK2 (Garrett and Collins, 2011; Nguyen and Tepe, 2011). Cloning of the CHK2 gene was first described by Matsuoka *et al.* (1998). ATM and ATR phosphorylate, CHK2 and CHK1 respectively, leading to activation of checkpoints. CHK2 acts as a signal distributor, dispersing checkpoint signal to downstream targets such as p53, Cdc25A, Cdc25C, BRCA1 and E2F1 (Perona *et al.*, 2008). In addition to its phosphorylation, CHK2 is also ubiquitylated, and both post-translational modifications are important for its function. The ubiquitin E3 ligase PIRH2 (p53-induced protein with a RING (Re-

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.92 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~89.5 kDa

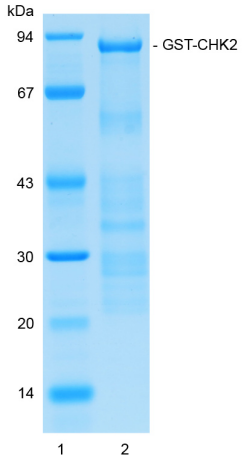
Purity: >85% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg GST-CHK2



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-CHK2 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-CHK2 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CHKtide substrate (250 µM) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-CHK2 specific activity:
621.6 Units/mg (571.8 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: CHKtide (KKKVSRSGLYRSPSMPENLNRPR)

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

ally Interesting New Gene)-H2 domain) interacts with CHK2 and mediates its polyubiquitylation and proteasomal degradation. The deubiquitylating enzyme involved in this process is USP28, forming a complex with PIRH2 and CHK2 and antagonizing PIRH2-mediated polyubiquitylation and proteasomal degradation of CHK2 (Bohgaki *et al.*, 2013). Inhibition of CHK2 is thought to sensitize p53-mutated or p53-deficient cancerous cells but protect normal tissue following DNA-damage caused by ionizing radiation or chemotherapeutic agents. The development of checkpoint kinase inhibitors for the treatment of cancer has therefore been a major objective in drug discovery over the past decade. Several inhibitors have been co-crystallized in the active site of CHK2 revealing important features of effective inhibitors. Some of these inhibitors have entered clinical trials in the last decade (Nguyen and Tepe, 2011).

References:

Bohgaki M, Hakem A, Halaby MJ, Bohgaki T, Li Q, Bissey PA, *et al.* (2013) The E3 ligase PIRH2 polyubiquitylates CHK2 and regulates its turnover. *Cell Death Differ* **20**, 812-822.

Garrett MD and Collins I (2011) Anticancer therapy with checkpoint inhibitors: what, where and when? *Trends Pharmacol Sci* **32**, 308-316.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Matsuoka S, Huang M and Elledge SJ (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* **282**, 1893-1897.

Nguyen TN and Tepe JJ (2011) Current inhibitors of checkpoint kinase 2. *Curr Med Chem* **18**, 4368-4374.

Perona R, Moncho-Amor V, Machado-Pinilla R, Belda-Iniesta C and Sanchez Perez I (2008) Role of CHK2 in cancer development. *Clin Transl Oncol* **10**, 538-542.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLVPRGSR
RASVGSHMPMSRPRRP***S***DVEAQQSHGSSAC
SQPHGSVTQSQGSSSQSQGISSSSTST
MPNSSQSSHSSSGTLSSLETVSTQELYSIP
EDQEPEDQEPEEPTPAPWARLWALQDGFAN
LECVNDNYWFGRDKSCEYCFDEPLLKRT
DKYRTYSKKHFRIFREVGPKNSYIAY
IEDHSGNGTFVNTELVGKGKRRPLNNNSE
IALSLSRNKVFVFFDLTVDDQSVYPKALRDEY
IMSKTLGSGACGEVKLAFERKTCKKVAIKI
ISKRKFAIGSAREADPALNVETEIEILKKL
NHPCIIKIKNFFDAEDYYIVLELMEGGELFD
KVVGNKRLKEATCKLYFYQMLLAVQYLHENGI
IHRDLKPENVLLSSQEEDCLIKITDFGHS
KILGETSLMRTLCGTPTYLAPEVLVSVGTAGY
NRAVDCWSLGVILFICLSGYPPFSEHRTQVS
LKDQITSGKYNFIPEVWAEVSEKALDLVKKLL
VVDPKARFTTEEALRHPWLQDEDMKRKFQDLL
SEENESTALPQVLAQPSTSRKRPREGEAE
GAETTKRPAVCAAVL**HHHHHH**

Tag (**bold text**): N-terminal GST and C-terminal 6His
Protease cleavage site: Thrombin (LVPR▼GS)
CHK2 (regular text): Start ***bold italics*** (amino acid residues 5-543.
Accession number: NP_009125

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