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Standard Operating Procedure

Preparation of active GST-CHK1

Enzyme description:-	GST-CHK1
Source:-	Recombinant
Expression system:-	Baculovirus expression vector system (BEVS)/Insect cells.
Tag:-	N-terminal GST
Purification method:-	GSH- agarose
Expression level:-	3-5 mg/L
Molecular mass:-	80 kDa
Purity:-	>80%
Contaminants:-	The preparation also contains 3 GSH-agarose binding proteins from insect cells, which migrate between 25 – 28kDa.
Activation protocol:-	N/A - constitutively active when purified from insect cells.

Enzyme storage buffer:-

50mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1mM EGTA, 0.1 % β-mercaptoethanol, 0.02 % Brij-35.

Storage temperature:- Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET –human CHK1

Protein	Human CHK1
Accession no	AF016582
Tags	GST amino terminal
Baculovirus-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDE GDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRY IADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAY SKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRI EAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKS DLEVLFQGPLGSPEF**MAVPFVEDWDLVQTLGEGAY GEVQLAVNRVTEEAVAVKIVDMKRAVDCPENIKK EICINKMLNHENVVKFYGHRREGNIQYLFLEYCSG GELFDRIEPDIGMPEPDAQRFFHQLMAGVVYLHGI GITHRDIKPENLLLDERDNLKISDFGLATVFRYNNR ERLLNKMCGTLPYVAPELLKRREFHAEPVDVWSCG IVLTAMLAGELPWDQPSDSCQEYSDWKEKKTYLN PWKKIDSAPLALLHKILVENPSARITIPDIKKDRWY NKPLKKGAKRPRVTSGGVSESPSGFSKHIQSNLDF SPVNSASSEENVKYSSSQPEPRTGLSLWDTSPSYID KLVQGISFSQPTCPDHMLLNSQLLGTPGSSQNPW QRLVKRMTRFFTKLDADKSYQCLKETCEKLGYQW KKSCMNQVTISTTDRRNNKLIFKVNLLEMDDKILV DFRLSKGDGLEFKRHFLKIKGKLIDIVSSQKVWLPA T***
Native sequence	1M of CHK1 is M235 of expressed protein
Protease cleavage site	Precission protease site (LEVLFQGP) residues 221-229 of expressed protein
Cloning sites	EcoR1, Not1 of pFBHTa

ORF in baculovirus

ATGGCAGTGCCCTTTGTGGAAGACTGGGACTTGGTGCAAACCCTGGGA
GAAGGTGCCTATGGAGAAGTTCAACTTGCTGTGAATAGAGTAACTGAA
GAAGCAGTCGCAGTGAAGATTGTAGATATGAAGCGTGCCGTAGACTGT
CCAGAAAATATTAAGAAAGAGATCTGTATCAATAAAATGCTAAATCAT
GAAAATGTAGTAAAATTCTATGGTCACAGGAGAGAAGGCAATATCCAA
TATTTATTTCTGGAGTACTGTAGTGGAGGAGAGCTTTTTGACAGAATA
GAGCCAGACATAGGCATGCCTGAACCAGATGCTCAGAGATTCTTCCAT
CAACTCATGGCAGGGGTGGTTTATCTGCATGGTATTGGAATAACTCAC
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