Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

CDC7/DBF4

cell division cycle 7

Recombinant Human Active Protein Kinase

HGNC Symbol: CDC7

Synonyms: CDC7L1, HsCdc7, HsCDC7, Hsk1, huCdc7

Product No.: 1513-1516-1

Lot: 006

Description: Human CDC7 amino acids M_1-L_{574} (as in NCBI/Protein entry NP_003494.1) N-terminal GST-HIS_6 fusion protein with a 3C cleavage site and DBF4, amino acids M_1-F_{674} (as in NCBI/Protein entry NP_006707.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, coexpressed in Sf9 insect cells

Product identity: CDC7/DBF4 Lot 006, was confirmed as CDC7/DBF4 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDCz}$: 91,978 Da
Theoretical $MW_{GST\text{-}DBF4}$: 104,938 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

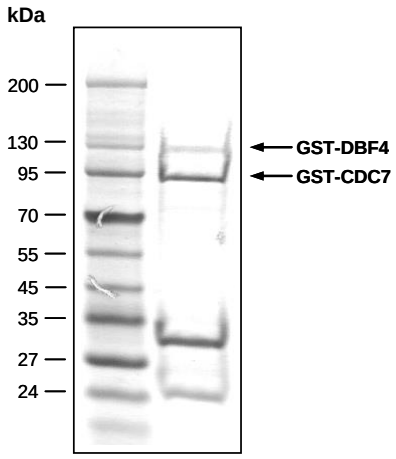
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.197 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

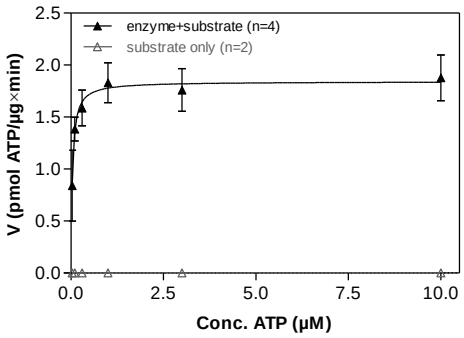
Biochemical Parameters:
Specific kinase activity (P_i transfer): 1.8 pmol/µg × min
ATP-K_M: 0.04 µM

CDC7/DBF4 Lot 006:
Coomassie stain



2.0 µg GST-CDC7/DBF4

CDC7/DBF4 Lot 006:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: Histone H1, 20 µg/ml
  CDC7/DBF4: 2 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
CDC7/DBF4 Lot 006 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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CDC7/DBF4

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GST-CDC7 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG	240
241	PMEASLGIQM DEPMAFSPQR DRFQAEGSLK KNEQNFKLAG VKKDIEKLYE AVPQLSNVFK	300
301	IEDKIGEGTF SSVYLATAQL QVGPEEKIAL KHLIPTSHPI RIAAELQCLT VAGGQDNVMG	360
361	VKYCFRKNDH VVIAMPYLEH ESFLDILNSL SFQEVREYML NLFKALKRIH QFGIVHRDVK	420
421	PSNFLYNRRL KKYALVDFGL AQGTHDTKIE LLKFVQSEAQ QERCSQNKSH IITGNKIPLS	480
481	GPVPKELDQQ STTKASVKRP YTNAQIQIKQ GKDGKEGSVG LSVQRSVFGE RNFNIHSSIS	540
541	HESPAVKLMK QSKTVDVLSR KLATKKKAIS TKVMNSAVMR KTASSCPASL TCDCYATDKV	600
601	CSICLSRRQQ VAPRAGTPGF RAPEVLTKCP NQTTAIDMWS AGVIFLSLLS GRYPFYKASD	660
661	DLTALAQIMT IRGSRETIQA AKTFGKSILC SKEVPAQDLR KLCERLRGMD SSTPKLTSDI	720
721	QGHASHQPAI SEKTDHKASC LVQTPPGQYS GNSFKKGDSN SCEHCFDEYN TNLEGWNEVP	780
781	DEAYDLLDKL LDLNPASRIT AEEALLHPFF KDMSL	840

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDC7

CDC7 wt[1] Amino Acid Sequence

1	MEASLGIQMD EPMAFSPQRD RFQAEGSLKK NEQNFKLAGV KKDIEKLYEA VPQLSNVFKI	60
61	EDKIGEGTFS SVYLATAQLQ VGPEEKIALK HLIPTSHPIR IAAELQCLTV AGGQDNVMGV	120
121	KYCFRKNDHV VIAMPYLEHE SFLDILNSLS FQEVREYMLN LFKALKRIHQ FGIVHRDVKP	180
181	SNFLYNRRLK KYALVDFGLA QGTHDTKIEL LKFVQSEAQQ ERCSQNKSHI ITGNKIPLSG	240
241	PVPKELDQQS TTKASVKRPY TNAQIQIKQG KDGKEGSVGL SVQRSVFGER NFNIHSSISH	300
301	ESPAVKLMKQ SKTVDVLSRK LATKKKAIST KVMNSAVMRK TASSCPASLT CDCYATDKVC	360
361	SICLSRRQQV APRAGTPGFR APEVLTKCPN QTTAIDMWSA GVIFLSLLSG RYPFYKASDD	420
421	LTALAQIMTI RGSRETIQAA KTFGKSILCS KEVPAQDLRK LCERLRGMDS STPKLTSDIQ	480
481	GHASHQPAIS EKTDHKASCL VQTPPGQYSG NSFKKGDSNS CEHCFDEYNT NLEGWNEVPD	540
541	EAYDLLDKLL DLNPASRITA EEALLHPFFK DMSL	600

blue: CDC7 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_003494.1

Recombinant Proteins

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Recombinant Proteins

GST-DBF4 Recombinant Fusion Protein Amino Acid Sequence

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241 PMNSGAMRIH SKGHFQGGIQ VKNEKNRPSL KSLKTDNRPE KSKCKPLWGK VFYLDLPSVT 300
301 ISEKLQKDIK DLGGRVEEFL SKDISYLISN KKEAKFAQTL GRISPVPSPE SAYTAETTSP 360
361 HPSHDGSSFK SPDTVCLSRG KLLVEKAIKD HDFIPSNSIL SNALSWGVKI LHIDDIRYYI 420
421 EQKKKELYLL KKSSTSVRDG GKRVGSGAQK TRTGRLKKPF VKVEDMSQLY RPFYLQLTNM 480
481 PFINYSIQKP CSPFDVDKPS SMQKQTQVKL RIQTDGDKYG GTSIQLQLKE KKKKGYCECC 540
541 LQKYEDLETH LLSEQHRNFA QSNQYQVVDD IVSKLVFDFV EYEKDTPKKK RIKYSVGSLS 600
601 PVSASVLKKT EQKEKVELQH ISQKDCQEDD TTVKEQNFLY KETQETEKKL LFISEPISHP 660
661 SNELRGLNEK MSNKCSMLST AEDDIRQNFT QLPLHKNKQE CILDISEHTL SENDLEELRV 720
721 DHYKCNIQAS VHVSDFSTDN SGSQPKQKSD TVLFPAKDLK EKDLHSIFTH DSGLITINSS 780
781 QEHLTVQAKA PFHTPPEEPN ECDFKNMDSL PSGKIHRKVK IILGRNRKEN LEPNAEFDKR 840
841 TEFITQEENR ICSSPVQSLL DLFQTSEEKS EFLGFTSYTE KSGICNVLDI WEEENSDNLL 900
901 TAFFSSPSTS TFTGF                                                  960
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: DBF4 **boxed**: variation from RefSeq

DBF4 wt[2] Amino Acid Sequence

```
1   MNSGAMRIHS KGHFQGGIQV KNEKNRPSLK SLKTDNRPEK SKCKPLWGKV FYLDLPSVTI  60
61  SEKLQKDIKD LGGRVEEFLS KDISYLISNK KEAKFAQTLG RISPVPSPES AYTAETTSPH 120
121 PSHDGSSFKS PDTVCLSRGK LLVEKAIKDH DFIPSNSILS NALSWGVKIL HIDDIRYYIE 180
181 QKKKELYLLK KSSTSVRDGG KRVGSGAQKT RTGRLKKPFV KVEDMSQLYR PFYLQLTNMP 240
241 FINYSIQKPC SPFDVDKPSS MQKQTQVKLR IQTDGDKYGG TSIQLQLKEK KKKGYCECCL 300
301 QKYEDLETHL LSEQHRNFAQ SNQYQVVDDI VSKLVFDFVE YEKDTPKKKR IKYSVGSLSP 360
361 VSASVLKKTE QKEKVELQHI SQKDCQEDDT TVKEQNFLYK ETQETEKKLL FISEPIPHPS 420
421 NELRGLNEKM SNKCSMLSTA EDDIRQNFTQ LPLHKNKQEC ILDISEHTLS ENDLEELRVD 480
481 HYKCNIQASV HVSDFSTDNS GSQPKQKSDT VLFPAKDLKE KDLHSIFTHD SGLITINSSQ 540
541 EHLTVQAKAP FHTPPEEPNE CDFKNMDSLP SGKIHRKVKI ILGRNRKENL EPNAEFDKRT 600
601 EFITQEENRI CSSPVQSLLD LFQTSEEKSE FLGFTSYTEK SGICNVLDIW EEENSDNLLT 660
661 AFFSSPSTST FTGF                                                  720
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blue: DBF4 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[2]NCBI/Protein accession number NP_006707.1