Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ GAK

cyclin G associated kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: GAK

Synonyms: Auxilin-2, DNAJC26

Product No.: 1974-0000-1

Lot: 003

Description: Human Kinase, N-terminal fragment, amino acids M_1-V_{400} (as in NCBI/Protein entry NP_005246.2), untagged, expressed in Sf9 insect cells

Product identity: GAK Lot 003, has been verified by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 44510 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

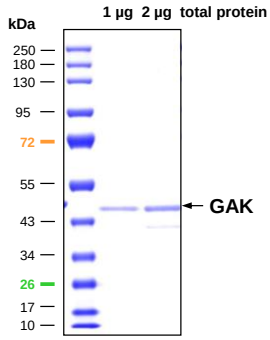
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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!

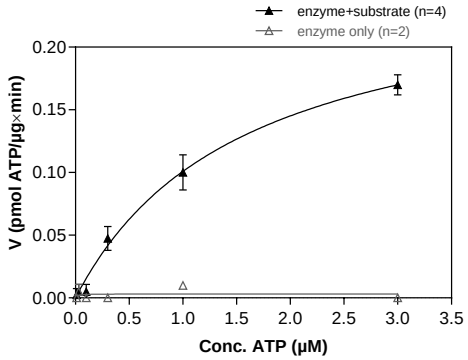
Protein concentration: 0.466 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 0.3 pmol/µg*min
ATP-K_M: 1.6 µM

GAK Lot 003:
Coomassie stain



GAK Lot 003:
Determination of V_{max} and K_M value for ATP



- 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: ERK2-K54R 80 µg/ml
  Kinase: 4 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Sequence information

GAK Recombinant Fusion Protein Amino Acid Sequence

1	GP**MSLLQSAL DFLAGPGSLG GASGRDQSDF VGQTVELGEL RLRVRRVLAE GGFAFVYEAQ**	60
61	**DVGSGREYAL KRLLSNEEEK NRAIIQEVCF MKKLSGHPNI VQFCSAASIG KEESDTGQAE**	120
121	**FLLLTELCKG QLVEFLKKME SRGPLSCDTV LKIFYQTCRA VQHMHRQKPP IIHRDLKVEN**	180
181	**LLLSNQGTIK LCDFGSATTI SHYPDYSWSA QRRALVEEEI TRNTTPMYRT PEIIDLYSNF**	240
241	**PIGEKQDIWA LGCILYLLCF RQHPFEDGAK LRIVNGKYSI PPHDTQYTVF HSLIRAMLQV**	300
301	**NPEERLSIAE VVHQLQEIAA ARNVNPKSPI TELLEQNGGY GSATLSRGPP PPVGPAGSGY**	360
361	**SGGLALAEYD QPYGGFLDIL RGGTERLFTN LKDTSSKVIQ SV**	420

1-2: leagcy of tag-cleavage **blue**: GAK fragment

GAK wt[1] Amino Acid Sequence

1	**MSLLQSALDF LAGPGSLGGA SGRDQSDFVG QTVELGELRL RVRRVLAEGG FAFVYEAQDV**	60
61	**GSGREYALKR LLSNEEEKNR AIIQEVCFMK KLSGHPNIVQ FCSAASIGKE ESDTGQAEFL**	120
121	**LLTELCKGQL VEFLKKMESR GPLSCDTVLK IFYQTCRAVQ HMHRQKPPII HRDLKVENLL**	180
181	**LSNQGTIKLC DFGSATTISH YPDYSWSAQR RALVEEEITR NTTPMYRTPE IIDLYSNFPI**	240
241	**GEKQDIWALG CILYLLCFRQ HPFEDGAKLR IVNGKYSIPP HDTQYTVFHS LIRAMLQVNP**	300
301	**EERLSIAEVV HQLQEIAAAR NVNPKSPITE LLEQNGGYGS ATLSRGPPPP VGPAGSGYSG**	360
361	**GLALAEYDQP YGGFLDILRG GTERLFTNLK DTSSKVIQSV** ANYAKGDLDI SYITSRIAVM	420
421	SFPAEGVESA LKNNIEDVRL FLDSKHPGHY AVYNLSPRTY RPSRFHNRVS ECGWAARRAP	480
481	HLHTLYNICR NMHAWLRQDH KNVCVVHCMD GRAASAVAVC SFLCFCRLFS TAEAAVYMFS	540
541	MKRCPPGIWP SHKRYIEYMC DMVAEEPITP HSKPILVRAV VMTPVPLFSK QRSGCRPFCE	600
601	VYVGDERVAS TSQEYDKMRD FKIEDGKAVI PLGVTVQGDV LIVIYHARST LGGRLQAKMA	660
661	SMKMFQIQFH TGFVPRNATT VKFAKYDLDA CDIQEKYPDL FQVNLEVEVE PRDRPSREAP	720
721	PWENSSMRGL NPKILFSSRE EQQDILSKFG KPELPRQPGS TAQYDAGAGS PEAEPTDSDS	780
781	PPSSSADASR FLHTLDWQEE KEAETGAENA SSKESESALM EDRDESEVSD EGGSPISSEG	840
841	QEPRADPEPP GLAAGLVQQD LVFEVETPAV LPEPVPQEDG VDLLGLHSEV GAGPAVPPQA	900
901	CKAPSSNTDL LSCLLGPPEA ASQGPPEDLL SEDPLLLASP APPLSVQSTP RGGPPAAADP	960
961	FGPLLPSSGN NSQPCSNPDL FGEFLNSDSV TVPPSFPSAH SAPPPSCSAD FLHLGDLPGE	1020
1021	PSKMTASSSN PDLLGGWAAW TETAASAVAP TPATEGPLFS PGGQPAPCGS QASWTKSQNP	1080
1081	DPFADLGDLS SGLQGSPAGF PPGGFIPKTA TTPKGSSSWQ TSRPPAQGAS WPPQAKPPPK	1140
1141	ACTQPRPNYA SNFSVIGARE ERGVRAPSFA QKPKVSENDF EDLLSNQGFS SRSDKKGPKT	1200
1201	IAEMRKQDLA KDTDPLKLKL LDWIEGKERN IRALLSTLHT VLWDGESRWT PVGMADLVAP	1260
1261	EQVKKHYRRA VLAVHPDKAA GQPYEQHAKM IFMELNDAWS EFENQGSRPL F	1320

blue: GAK sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005246.2

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