KinaseDetect ApS
http://www.kinasedetect.com
VAT nr. DK 26 40 38 04

Technical support:
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Certificate of Analysis

Product	**AMP-activated protein kinase $\alpha_1/\beta_1/\gamma_1$ (AMPKa1/b1/g1), human recombinant, expressed in *E. coli***
Cat No	PK-035-01
Lot No	021014

Description

The human AMPK subunits α_1, β_1 and γ_1 were co-expressed in *E.coli* [1]. The α_1-subunit has a N-terminal maltose binding protein (MBP) tag. The heterotrimeric AMPK complex was purified using an amylose matrix. The catalytic subunit α_1 was activated by *in vitro* phosphorylation using CAMKK2. CAMKK2 and impurities were removed using HiLoad 16/600 Superdex 200 prep grade for size exclusion chromatography. The MBP-tag can be removed using TEV protease.

Approved HUGO gene symbols: AMPK α_1: PRKAA1; AMPK β_1: PRKAB1; AMPK γ_1: PRKAG1

Synonyms: 5' adenosine monophosphate-activated protein kinase

Quality

Protein concentration (Bradford with BSA as standard)	1.5 mg/ml
Purity	> 95 % by SDS PAGE
Specific activity	4.300.000*/ mg
* 1 Unit is defined as 1 picmole phosphate transferred to synthetic peptide AMARA (AMARAASAAALARRR) per min at 30 °C	
Protease activity (Twining test)	none

Form Liquid. 200 mM NaCl; 10 % (v/v) glycerol; 1 mM PMSF; 0.5 mM TCEP; 50 mM Tris /HCl pH 8.0, Complete (Roche, 1 tablet/50 ml)

Package size 20 microgram

Storage condition -70 °C

Shipment conditions dry ice

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

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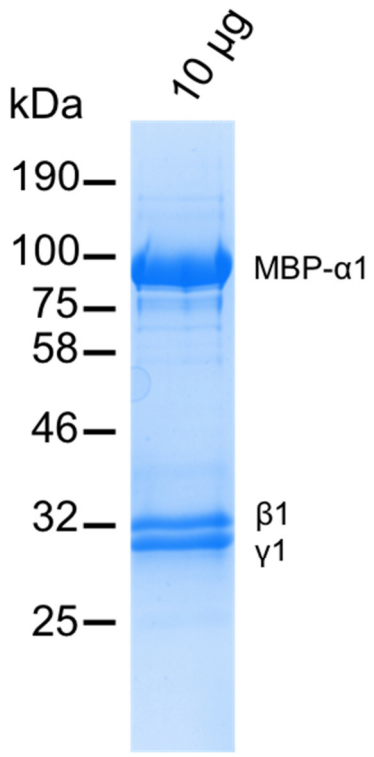
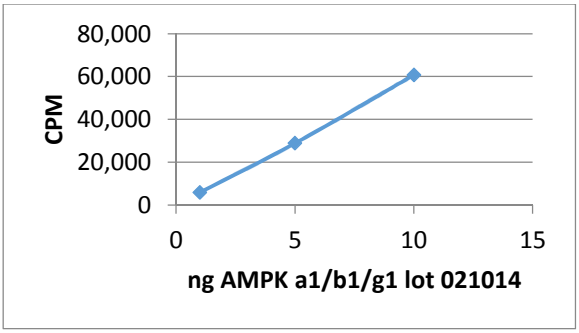
SDS-PAGE analysis



(Note: For AMPKβ1 is an anomalous electrophoretic mobility described in [2])

Activity Assay



Amino acid sequence information

MBP-AMPK α_1: M393 corresponds to M1 of BC048980. Sequence based calculated M.W.: 105838.3 Da; TEV-cleavage site is underlined.

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MKIEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ 50
VAATGDGPDI IFWAHDRFGG YAQSGLLAEI TPDKAFQDKL YPFTWDAVRY 100
NGKLIAYPIA VEALSLIYNK DLLPNPPKTW EEIPALDKEL KAKGKSALMF 150
NLQEPYFTWP LIAADGGYAF KYENGKYDIK DVGVDNAGAK AGLTFLVDLI 200
KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK VNYGVTVLPT 250
FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL 300
GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN 350
AASGRQTVDE ALKDAQTNSS LNNNNNNNNN NLGENLYFQG GSMATAEKQK 400
HDGRVKIGHY ILGDTLGVGT FGKVKVGKHE LTGHKVAVKI LNRQKIRSLD 450
VVGKIRREIQ NLKLFRHPHI IKLYQVISTP SDIFMVMEYV SGGELFDYIC 500
KNGRLDEKES RRLFQQILSG VDYCHRHMVV HRDLKPENVL LDAHMNAKIA 550
DFGLSNMMSD GEFLRTSCGS PNYAAPEVIS GRLYAGPEVD IWSSGVILYA 600
LLCGTLPFDD DHVPTLFKKI CDGIFYTPQY LNPSVISLLK HMLQVDPMKR 650
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ATIKDIREHE WFKQDLPKYL FPEDPSYSST MIDDEALKEV CEKFECSEEE 700
VLSCLYNRNH QDPLAVAYHL IIDNRRIMNE AKDFYLATSP PDSFLDDHHL 750
TRPHPERVPF LVAETPRARH TLDELNPQKS KHQGVRKAKW HLGIRSQSRP 800
NDIMAEVCRA IKQLDYEWKV VNPYYLRVRR KNPVTSTYSK MSLQLYQVDS 850
RTYLLDFRSI DDEITEAKSG TATPQRSGSV SNYRSCQRSD SDAEAQGKSS 900
EVSLTSSVTS LDSSPVDLTP RPGSHTIEFF EMCANLIKIL AQ         942
```

AMPK β_1: M1 corresponds to M1 of AJ224515.
Sequence based calculated M.W.: 30382.3 Da

```
MGNTSSERAA LERHGGHKTP RRDSSGGTKD GDRPKILMDS PEDADLFHSE 50
EIKAPEKEEF LAWQHDLEVN DKAPAQARPT VFRWTGGGKE VYLSGSFNNW 100
SKLPLTRSHN NFVAILDLPE GEHQYKFFVD GQWTHDPSEP IVTSQLGTVN 150
NIIQVKKTDF EVFDALMVDS QKCSDVSELS SSPPGPYHQE PYVCKPEERF 200
RAPPILPPHL LQVILNKDTG ISCDPALLPE PNHVMLNHLY ALSIKDGVMV 250
LSATHRYKKK YVTTLLYKPI                                  270
```

AMPK γ_1: M1 corresponds to M1 of U42412
Sequence based calculated M.W.: 37579.3 Da

```
METVISSDSS PAVENEHPQE TPESNNSVYT SFMKSHRCYD LIPTSSKLVV 50
FDTSLQVKKA FFALVTNGVR AAPLWDSKKQ SFVGMLTITD FINILHRYYK 100
SALVQIYELE EHKIETWREV YLQDSFKPLV CISPNASLFD AVSSLIRNKI 150
HRLPVIDPES GNTLYILTHK RILKFLKLFI TEFPKPEFMS KSLEELQIGT 200
YANIAMVRTT TPVYVALGIF VQHRVSALPV VDEKGRVVDI YSKFDVINLA 250
AEKTYNNLDV SVTKALQHRS HYFEGVLKCY LHETLETIIN RLVEAEVHRL 300
VVVDENDVVK GIVSLSDILQ ALVLTGGEKK P                      331
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In vitro Kinase Assay

Assay Components

Assay Buffer (AB): 100 mM HEPES pH 7,5, 40 mM $MgCl_2$, 2 mM Na_3VO_4, 20 mM β-glycerophosphate, 0.04% CHAPS, 10 mM DTT, 600 mM AMP, Complete (Roche, 1 tablet/50 ml)
Dilution Buffer (DB): 20 mM Tris/HCl pH7,5, 0.01% CHAPS, 0.5 mM, Na_3VO_4, 2 mM DTT, 10% glycerol
Substrate: AMPK peptide AMARA, 800 µM.
Protein kinase: AMPK $\alpha_1/\beta_1/\gamma_1$ Lot No 091014, 1,5 mg/mL, diluted to 0.5-1 ng/µL in DB directly before use.
Magnesium/ATP Cocktail: 75 mM $MgCl_2$, 500 µM ATP
Diluted [γ–^{32}P]ATP: Mix 197 µL Magnesium/ATP Cocktail with 3 µL(30 µCi) [γ–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay Procedure

All compounds are pipetted into a microcentrifuge tube on ice
1. Add 10 µL AB.
2. Add 10 µL 800 microM AMARA peptide.
3. Add 10 µL diluted AMPK $\alpha_1/\beta_1/\gamma_1$, 5 – 10 ng/assay.
4. Add 10 µL of the diluted [γ–^{32}P]ATP.
5. Incubate 10 min at 30 ^{o}C.
6. Stop the reaction by setting samples on ice.
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec).
8. Immerse the paper in 85mM phosphoric acid. Shake gently on a rotator.
9. Wash 3 times for 5 minutes with phosphoric acid.
10. Wash briefly with acetone.
11. Dry under infrared light.
12. Count ina liquid scintillation counter.

References

[1] Tan, S. (2001) A Modular Polycistronic Expression System for Overexpressing Protein Complexes in *Escherichia coli* Prot Expr Pur 21, 224-234

[2] Neumann, D.; Woods, A.; Carling, D; Wallimann, T. and Uwe Schlattner, U. (2003) Mammalian AMP-activated protein kinase: functional, heterotrimeric complexes by co-expression of subunits in *Escherichia coli*□ Prot Expr Pur 30, 230–237