

Certificate of Analysis

AMPK ($\alpha 1$, $\beta 1$, $\gamma 1$), active
(Recombinant enzyme expressed in *E. coli*)
Item # 14-840, 14-840-K, 14-840M
Parent Lot # 1814904

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Recombinant, human AMPK ($\alpha 1$, $\beta 1$, $\gamma 1$) holoenzyme, comprised of full length N-terminal 6His-tagged AMPK $\alpha 1$, full length untagged AMPK $\beta 1$ and full length untagged AMPK $\gamma 1$. Expressed in *E. coli* cells and activated *in vivo*. Purified using Ni²⁺/NTA agarose.

Purity 58.8% by SDS-PAGE and Coomassie blue staining. AMPK $\alpha 1$ MW = 64kDa, AMPK $\beta 1$ MW = 30kDa, AMPK $\gamma 1$ MW = 38kDa

Specific Activity (Parent lot# 1814904): 3099U/mg, where one unit of AMPK ($\alpha 1$, $\beta 1$, $\gamma 1$), active activity is defined as 1nmol phosphate incorporated into 50 μ M (AMARAASAAALARRR) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.427mg/ml of enzyme in 50mM Tris/HCl pH8.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

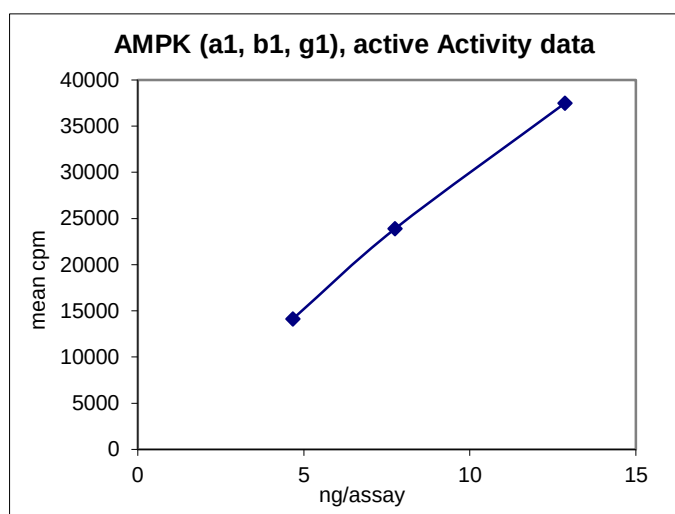
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

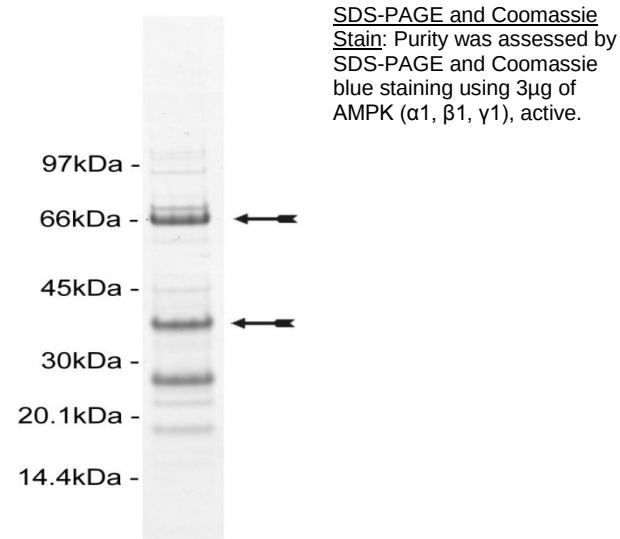
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 5–13ng of this lot of enzyme phosphorylated 50 μ M (AMARAASAAALARRR) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as AMPK ($\alpha 1$, $\beta 1$, $\gamma 1$) with the AMPK $\alpha 1$, AMPK $\beta 1$ and AMPK $\gamma 1$ translated sequences listed on pages three to five.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA, other components.
2. **(AMARAASAAALARRR):** Use at a final assay concentration of 50 μ M. Prepare a 500 μ M stock and add 2.5 μ l of stock per assay point.
3. **AMP:** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock and add 2.5 μ l per assay point.
4. **AMPK (α 1, β 1, γ 1), active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–13ng per assay point.
5. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(AMARAASAAALARRR)**.
3. Add **2.5 μ l (5–13ng) AMPK (α 1, β 1, γ 1)**.
4. Add 2.5 μ l of 2mM AMP.
5. Add 2.5 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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AMPK α 1 Sequence Information

<u>Protein</u>	Human AMPK α 1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human AMPK α 1
<u>Accession number</u>	GenBank AF100763

Recombinant AMPK α 1 amino acid sequence:

```

1  MHHHHHHMAT AEKQKHDGRV KIGHYILGDT LGVGTFGKVK VGKHELTGHK VAVKILNRQK
61  IRSLDVVGKI RREIQNLKLF RHPHIKLYQ VISTPSDIFM VMEYVSGGEL FDYICKNGRL
121 DEKESRRLFQ QILSGVDYCH RHMVVHRDLK PENVLLDAHM NAKIADFGLS NMMSDGEFLR
181 TSCGSPNYAA PEVISGRLYA GPEVDIWSSG VILYALLCGT LPFDDDHVPT LFKKICDGIF
241 YTPQYLNPSV ISLLKHMLQV DPMKRATIKD IREHEWFKQD LPKYLFPEDP SYSSTMIDDE
301 ALKEVCEKFE CSEEEVLSCS YNRNHQDPLA VAYHLIIDNR RIMNEAKDFY LATSPDPSFL
361 DDHHLTRPHP ERVPFLVAET PRARHTLDEL NPQKSKHQGV RKAKWHLGIR SQSRPNNDIMA
421 EVCRAIKQLD YEWKVVNPYY LRVRRKNPVT STYSKMSLQL YQVDSRTYLL DFRSIDDEIT
481 EAKSGTATPQ RSGSVSNYRS CQRSDSDAEA QGKSSEVSLT SSVTSLDSSP VDLTPRPGSH
541 TIEFFEMCAN LIKILAQ

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Recombinant AMPK α 1 nucleotide sequence:

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1  atgcaccatc accatcacca tatggcgaca gccgagaagc agaaacacga cgggcggggtg
61  aagatcggcc actacattct gggtgacacg ctgggggtcg gcaccttcgg caaagtgaag
121 gttggcaaac atgaattgac tgggcataaa gtagctgtga agatactcaa tcgacagaag
181 attcggagcc ttgatgtggt aggaaaaatc cgcagagaaa ttcagaacct caagcttttc
241 aggcattctc atataattaa actgtaccag gtcattcagta caccattctga ttttttcattg
301 gtgatggaat atgtctcagg aggagagcta tttgattata tctgtaagaa tgggaaggctg
361 gatgaaaaag aaagtcggcg tctgttccaa cagatccttt ctgggtgtgga ttattgtcac
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481 aatgcaaaga tagctgattt tgggtcttca aacatgatgt cagatgggtga atttttaaga
541 acaagtgtgt gctcacccaa ctatgctgca ccagaagtaa tttcaggaag attgtatgca
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661 cttccatttg atgatgacca tgtgccaact ctttttaaga agatatgtga tgggatcttc
721 tatacccttc aatattttaa tccttctgtg attagccttt tgaaacatat gctgcagggtg
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901 gccttaaaaag aagtattgtg aaagtttgag tgctcagaag aggaagttct cagctgtctt
961 tacaacagaa atcaccagga tcctttggca gttgcctacc atctcataat agataacagg
1021 agaataatga atgaagccaa agatttctat ttggcgacaa gccacctga ttcttttctt
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1141 ccaagggcac gccataccct tgatgaatta aatccacaga aatccaaaca ccaaggtgta
1201 aggaaagcaa aatggcattt aggaattaga agtcaaagtc gaccaaatga tattatggca
1261 gaagtattga gagcaatcaa acaattggat tatgaatgga aggttgtaaa cccatattat
1321 ttgctgttac gaaggaagaa tcctgtgaca agcacttact ccaaaatgag tctacagtta
1381 taccaagtgg atagtagaac ttatctactg gatttccgta gtattgatga tgaaattaca
1441 gaagccaaat cagggactgc tactccacag agatcgggat cagttagcaa ctatcgatct
1501 tgccaaagga gtgattcaga tgctgaggct caaggaaaat cctcagaagt ttctcttacc
1561 tcatctgtga cctcacttga ctcttctcct gttgacctaa ctccaagacc tgggaagtcac
1621 acaatagaat tttttgagat gtgtgcaaat ctaattaaaa ttcttgacaca ataa

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AMPK β 1 Sequence Information

<u>Protein</u>	Human AMPK β 1
<u>Tags</u>	untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human AMPK β 1
<u>Accession number</u>	GenBank NM_006253

Recombinant AMPK β 1 amino acid sequence:

```

1  MGNTSSERAA  LERHGGHKTP  RRDSSGGTKD  GDRPKILMDS  PEDADLFHSE  EIKAPEKEEF
61  LAWQHDLEVN  DKAPAQARPT  VFRWTGGGKE  VYLSGSFNW  SKLPLTRSHN  NFVAILDLPE
121  GEHQYKFFVD  GQWTHDPSEP  IVTSQLGTVN  NIIQVKKTD  EVFDALMVDS  QKCSDVSELS
181  SSPPGPYHQE  PYVCKPEERF  RAPPILPPHL  LQVILNKDTG  ISCDPALLPE  PNHVMLNHLY
241  ALSIKDGVMV  LSATHRYKKK  YVTTLKYKPI

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Recombinant AMPK β 1 nucleotide sequence:

```

1  atgggcaata  ccagcagtga  gcgcgccgcg  ctggagcggc  atggtggcca  taagacgccc
61  cggagggaca  gctcgggggg  caccaaggac  ggggacaggc  ccaagatcct  gatggacagc
121  cccgaagacg  ccgacctctt  ccaactccgag  gaaatcaagg  caccagagaa  ggaggaattc
181  ctggcctggc  agcatgatct  ggaagtgaat  gataaagctc  ccgcccaggc  tcggccaacg
241  gtgtttcgat  ggacgggggg  cggaaaggaa  gtttacttat  ctgggtcctt  caacaactgg
301  agtaaaacttc  ccctcaccag  aagccacaat  aactttgtag  ccatcctgga  tctgccggaa
361  ggagagcatc  agtacaagtt  ctttgtggat  ggtcagtgga  cgcacgaccc  ttccgagccc
421  atagtaacca  gccagcttgg  cacagttaac  aacatcattc  aagtgaagaa  aactgacttt
481  gaagtatttg  atgctttaat  ggtggattcc  caaaagtgct  ccgatgtgtc  tgagctgtcc
541  agttctcccc  caggacccta  ccatcaggag  ccctacgtct  gcaaaccgga  agagcgcttt
601  cgggcacccc  ctattctccc  cccacatctc  ctccaggtca  tcctgaacaa  ggacacgggg
661  atttcctgtg  atccagcttt  gcttcctgag  cccaatcacg  tcatgttgaa  ccacctatac
721  gcgctgtcta  tcaaggatgg  agtgatgggt  ctcagcgcaa  cccaccggta  caagaagaag
781  tacgtcacca  ccttggtata  caagcccata  tga

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AMPKy1 Sequence Information

<u>Protein</u>	Human AMPKy1
<u>Tags</u>	untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human AMPKy1
<u>Accession number</u>	GenBank NM_002733

Recombinant AMPKy1 amino acid sequence:

```

1  METVISSDSS  PAVENEHPQE  TPESNNSVYT  SFMKSHRCYD  LIPTSSKLTV  FDTSLQVKKA
61  FFALVTNGVR  AAPLWDSKKQ  SFVGMLTITD  FINILHRYRK  SALVQIYELE  EHKIETWREV
121 YLQDSFKPLV  CISPNASLFD  AVSSLIRNKI  HRLPVIDPES  GNTLYILTHK  RILKFLKLFI
181 TEFPKPEFMS  KSLEELQIGT  YANIAMVRTT  TPVYVALGIF  VQHRVSALPV  VDEKGRVVDI
241 YSKFDVINLA  AEKTYNNLDV  SVTKALQHRH  HYFEGVLKCY  LHETLETIIN  RLVEAEVHRL
301 VVVDENDVVK  GIVSLSDILQ  ALVLTGGEKK  P

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Recombinant AMPKy1 nucleotide sequence:

```

1  atggagacgg  tcatttcttc  agatagctcc  ccagctgtgg  aaaatgagca  tcctcaagag
61  accccagaat  ccaacaatag  cgtgtatact  tccttcatga  agtctcatcg  ctgctatgac
121 ctgattccca  caagctccaa  attggttgta  tttgatacgt  ccctgcaggt  gaagaaagct
181 ttttttgctt  tgggtgactaa  cgggtgtacga  gctgcccctt  tatgggatag  taagaagcaa
241 agttttgtgg  gcatgctgac  catcactgat  ttcatacaata  tcctgcaccg  ctactataaa
301 tcagccttgg  tacagatcta  tgagctagaa  gaacacaaga  tagaaacttg  gagagagggtg
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601 tatgccaata  ttgctatggg  tcgcactacc  acccccgctc  atgtggctct  ggggattttt
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841 ctgcatgaga  ctctggagac  catcatcaac  aggctagtgg  aagcagaggt  tcaccgactt
901 gtagtggtgg  atgaaaatga  tgtggtcaag  ggaattgtat  cactgtctga  catcctgcag
961 gccctggtgc  tcacaggtgg  agagaagaag  ccctga

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