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Certificate of Analysis

AMPK (α1, β1, γ1), 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 (α1, β1, γ1) holoenzyme, comprised of full length N-terminal 6His-tagged AMPKα1, full length untagged AMPKβ1 and full length untagged AMPKγ1. Expressed in *E coli* cells and activated *in vivo*. Purified using Ni^{2+}/NTA agarose.
Purity 58.8% by SDS-PAGE and Coomassie blue staining. AMPKα1 MW = 64kDa, AMPKβ1 MW = 30kDa, AMPKγ1 MW = 38kDa

Specific Activity (Parent lot# 1814904): 3099U/mg, where one unit of AMPK (α1, β1, γ1) activity is defined as 1nmol phosphate incorporated into 50µM (AMARAASAAALARRR) per minute at 30°C with a final ATP concentration of 100µM.

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.

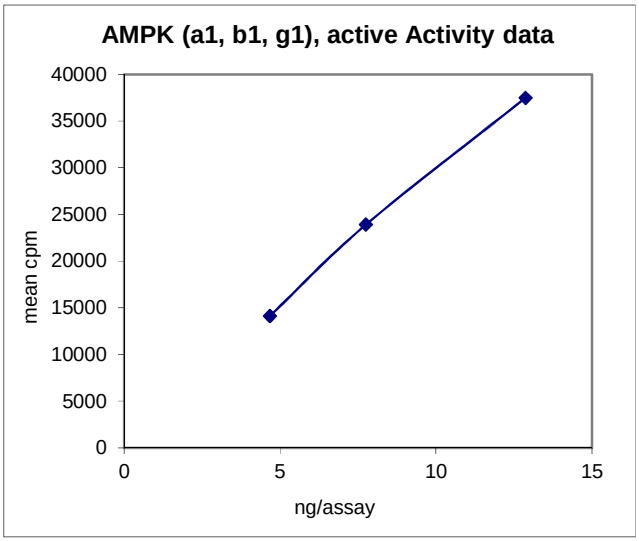
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

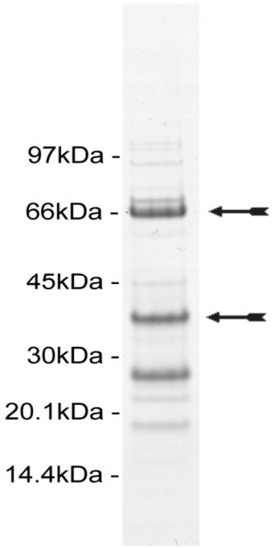
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 5–13ng of this lot of enzyme phosphorylated 50µM (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 (α1, β1, γ1) with the AMPKα1, AMPKβ1 and AMPKγ1 translated sequences listed on pages three to five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active AMPK (α1, β1, γ1).

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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 50µM. Prepare a 500µM stock and add 2.5µl of stock per assay point.
3. **AMP:** Use at a final assay concentration of 200µM. Prepare a 2mM stock and add 2.5µl per assay point.
4. **AMPK (α1, β1, γ1), 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-^{33}P]ATP:** 2.5 x MgAc/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(AMARAASAAALARRR)**.
3. Add **2.5µl (5–13ng) AMPK (α1, β1, γ1)**.
4. Add 2.5µl of 2mM AMP.
5. Add 2.5µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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AMPKα1 Sequence Information

Protein	Human AMPKα1
Tags	*N*-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human AMPKα1
Accession number	GenBank AF100763

Recombinant AMPKα1 amino acid sequence:

```
  1 MHHHHHHMAT AEKQKHDGRV KIGHYILGDT LGVGTFGKVK VGKHELTGHK VAVKILNRQK
 61 IRSLDVVGKI RREIQNLKLF RHPHIIKLYQ 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 CSEEEVLSCL YNRNHQDPLA VAYHLIIDNR RIMNEAKDFY LATSPPDSFL
361 DDHHLTRPHP ERVPFLVAET PRARHTLDEL NPQKSKHQGV RKAKWHLGIR SQSRPNDIMA
421 EVCRAIKQLD YEWKVVNPYY LRVRRKNPVT STYSKMSLQL YQVDSRTYLL DFRSIDDEIT
481 EAKSGTATPQ RSGSVSNYRS CQRSDSDAEA QGKSSEVSLT SSVTSLDSSP VDLTPRPGSH
541 TIEFFEMCAN LIKILAQ
```

Recombinant AMPKα1 nucleotide sequence:

```
   1 atgcaccatc accatcacca tatggcgaca gccgagaagc agaaacacga cgggcgggtg
  61 aagatcggcc actacattct gggtgacacg ctgggggtcg gcaccttcgg caaagtgaag
 121 gttggcaaac atgaattgac tgggcataaa gtagctgtga agatactcaa tcgacagaag
 181 attcggagcc ttgatgtggt aggaaaaatc cgcagagaaa ttcagaacct caagcttttc
 241 aggcatcctc atataattaa actgtaccag gtcatcagta caccatctga tattttcatg
 301 gtgatggaat atgtctcagg aggagagcta tttgattata tctgtaagaa tggaaggctg
 361 gatgaaaaag aaagtcggcg tctgttccaa cagatccttt ctggtgtgga ttattgtcac
 421 aggcatatgg tggtccatag agatttgaaa cctgaaaatg tcctgcttga tgcacacatg
 481 aatgcaaaga tagctgattt tggtctttca aacatgatgt cagatggtga atttttaaga
 541 acaagttgtg gctcacccaa ctatgctgca ccagaagtaa tttcaggaag attgtatgca
 601 ggcccagagg tagatatatg gagcagtggg gttattctct atgctttatt atgtggaacc
 661 cttccatttg atgatgacca tgtgccaact ctttttaaga agatatgtga tgggatcttc
 721 tatacccctc aatatttaaa tccttctgtg attagccttt tgaaacatat gctgcaggtg
 781 gatcccatga agagggccac aatcaaagat atcagggaac atgaatggtt taaacaggac
 841 cttccaaaat atctctttcc tgaggatcca tcatatagtt caaccatgat tgatgatgaa
 901 gccttaaaag aagtatgtga aaagtttgag tgctcagaag aggaagttct cagctgtctt
 961 tacaacagaa atcaccagga tcctttggca gttgcctacc atctcataat agataacagg
1021 agaataatga atgaagccaa agatttctat ttggcgacaa gcccacctga ttcttttctt
1081 gatgatcatc acctgactcg gccccatcct gaaagagtac cattcttggt tgctgaaaca
1141 ccaagggcac gccataccct tgatgaatta aatccacaga aatccaaaca ccaaggtgta
1201 aggaaagcaa aatggcattt aggaattaga agtcaaagtc gaccaaatga tattatggca
1261 gaagtatgta gagcaatcaa acaattggat tatgaatgga aggttgtaaa cccatattat
1321 ttgcgtgtac 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 tggaagtcac
1621 acaatagaat tttttgagat gtgtgcaaat ctaattaaaa ttcttgcaca ataa
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AMPKβ1 Sequence Information

Protein	Human AMPKβ1
Tags	untagged
Native sequence	M1 of the recombinant protein is equivalent to M1 of human AMPKβ1
Accession number	GenBank NM_006253

Recombinant AMPKβ1 amino acid sequence:

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

Recombinant AMPKβ1 nucleotide sequence:

```
  1 atgggcaata ccagcagtga gcgcgccgcg ctggagcggc atggtggcca taagacgccc
 61 cggagggaca gctcgggggg caccaaggac ggggacaggc ccaagatcct gatggacagc
121 cccgaagacg ccgacctctt ccactccgag gaaatcaagg caccagagaa ggaggaattc
181 ctggcctggc agcatgatct ggaagtgaat gataaagctc ccgcccaggc tcggccaacg
241 gtgtttcgat ggacgggggg cggaaaggaa gtttacttat ctgggtcctt caacaactgg
301 agtaaacttc 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 gcaaacccga agagcgcttt
601 cgggcacccc ctattctccc cccacatctc ctccaggtca tcctgaacaa ggacacgggg
661 atttcctgtg atccagcttt gcttcctgag cccaatcacg tcatgctgaa ccacctatac
721 gcgctgtcta tcaaggatgg agtgatggtg ctcagcgcaa cccaccggta caagaagaag
781 tacgtcacca ccttgttata caagcccata tga
```

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AMPKγ1 Sequence Information

Protein	Human AMPKγ1
Tags	untagged
Native sequence	M1 of the recombinant protein is equivalent to M1 of human AMPKγ1
Accession number	GenBank NM_002733

Recombinant AMPKγ1 amino acid sequence:

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

Recombinant AMPKγ1 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 tggtgactaa cggtgtacga gctgcccctt tatgggatag taagaagcaa
241 agttttgtgg gcatgctgac catcactgat ttcatcaata tcctgcaccg ctactataaa
301 tcagccttgg tacagatcta tgagctagaa gaacacaaga tagaaacttg gagagaggtg
361 tatctccagg actcctttaa accgcttgtc tgcatttctc ctaatgccag cttgtttgat
421 gctgtctctt cattaattcg gaacaagatc cacaggctgc cagttattga cccagaatca
481 ggcaatactt tgtacatcct cacccacaag cgcattctga agttcctcaa attgtttatc
541 actgagttcc ccaagccaga gttcatgtcc aagtctctgg aagagctaca gattggcacc
601 tatgccaata ttgctatggt tcgcactacc acccccgtct atgtggctct ggggattttt
661 gtacagcatc gagtctcagc cctgccagtg gtggatgaga aggggcgtgt ggtggacatc
721 tactccaagt ttgatgttat caatctggca gcagaaaaga cctacaacaa cctagatgta
781 tctgtgacga aagccttgca acatcgatca cattactttg agggtgttct caagtgctac
841 ctgcatgaga ctctggagac catcatcaac aggctagtgg aagcagaggt tcaccgactt
901 gtagtggtgg atgaaaatga tgtggtcaag ggaattgtat cactgtctga catcctgcag
961 gccctggtgc tcacaggtgg agagaagaag ccctga
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Reviewed and approved by site quality representative.

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