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Certificate of Analysis

AMPK (α2, β1, γ1), active

(Recombinant enzyme expressed in *E. coli*)
Item # 14-902, 14-902-K, 14-902M
Parent Lot # D8PN063N

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 (α2, β1, γ1) holoenzyme, comprised of full length N-terminal 6His-tagged AMPKα2, full length untagged AMPKβ1 and full length untagged AMPKγ1. Expressed in *E. coli* cells and activated *in vivo*. Purified using cobalt agarose.
Purity (α2, β1, γ1 subunits combined) 94% by SDS-PAGE and Coomassie blue staining. AMPKα2 MW = 63kDa, AMPKβ1 MW = 30kDa, AMPKγ1 MW = 38kDa

Specific Activity (Parent lot# D8PN063N): 2730U/mg, where one unit of AMPK (α2, β1, γ1), active activity is defined as 1nmol phosphate incorporated into 100µM (AMARAASAAALARRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.54mg/ml of enzyme in 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

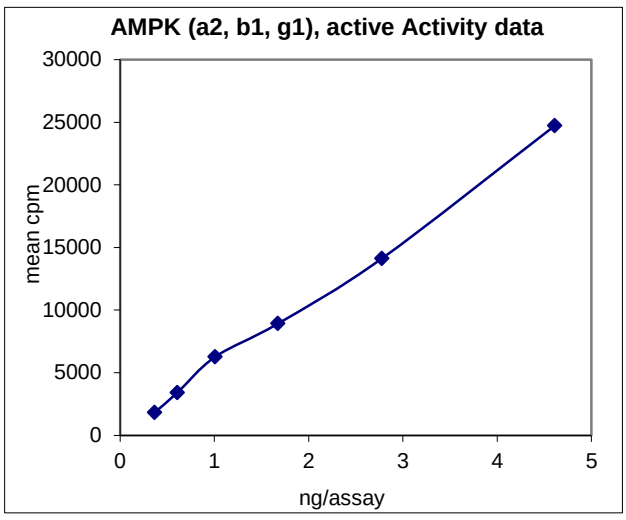
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

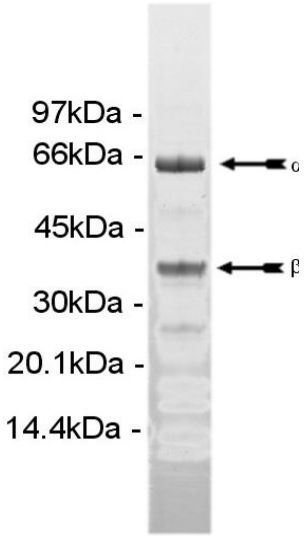
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.37–4.6ng of this lot of enzyme phosphorylated 100µM (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 product identity as AMPK (α2, β1, γ1) with the AMPKα2, 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 AMPK (α2, β1, γ1), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(AMARAASAAALARRR):** Use at a final assay concentration of 100µM. Prepare a 1mM stock and add 2.5µl of stock per assay point.
3. **AMP:** Use at a final assay concentration of 200µM. Make a 2mM stock. Add 2.5µl of stock per assay point.
4. **AMPK (α2, β1, γ1), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.37–4.6ng 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 (0.37–4.6ng) AMPK (α2, β1, γ1) active.**
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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AMPKα2 Sequence Information

Protein Human AMPKα2

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human AMPKα2

Accession number GenBank NM_006252

Recombinant AMPKα2 amino acid sequence:

```
   1 MHHHHHHMAE KQKHDGRVKI GHYVLGDTLG VGTFGKVKIG EHQLTGHKVA VKILNRQKIR
  61 SLDVVGKIKR EIQNLKLFRH PHIIKLYQVI STPTDFFMVM EYVSGGELFD YICKHGRVEE
 121 MEARRLFQQI LSAVDYCHRH MVVHRDLKPE NVLLDAHMNA KIADFGLSNM MSDGEFLRTS
 181 CGSPNYAAPE VISGRLYAGP EVDIWSCGVI LYALLCGTLP FDDEHVPTLF KKIRGGVFYI
 241 PEYLNRSVAT LLMHMLQVDP LKRATIKDIR EHEWFKQDLP SYLFPEDPSY DANVIDDEAV
 301 KEVCEKFECT ESEVMNSLYS GDPQDQLAVA YHLIIDNRRI MNQASEFYLA SSPPSGSFMD
 361 DSAMHIPPGL KPHPERMPPL IADSPKARCP LDALNTTKPK SLAVKKAKWH LGIRSQSKPY
 421 DIMAEVYRAM KQLDFEWKVV NAYHLRVRRK NPVTGNYVKM SLQLYLVDNR SYLLDFKSID
 481 DEVVEQRSGS STPQRSCSAA GLHRPRSSFD STTAESHSLS GSLTGSLTGS TLSSVSPRLG
 541 SHTMDFFEMC ASLITTLAR
```

Recombinant AMPKα2 nucleotide sequence:

```
   1 atgcaccatc accatcacca tatggctgag aagcagaagc acgacgggcg ggtgaagatc
  61 ggacactacg tgctgggcga cacgctgggc gtcggcacct tcggcaaagt gaagattgga
 121 gaacatcaat taacaggcca taaagtggca gttaaaatct taaatagaca gaagattcgc
 181 agtttagatg ttgttggaaa aataaaacga gaaattcaaa atctaaaact ctttcgtcat
 241 cctcatatta tcaaactata ccaggtgatc agcactccaa cagatttttt tatggtaatg
 301 gaatatgtgt ctggaggtga attatttgac tacatctgta agcatggacg ggttgaagag
 361 atggaagcca ggcggctctt tcagcagatt ctgtctgctg tggattactg tcataggcat
 421 atggttgttc atcgagacct gaaaccagag aatgtcctgt tggatgcaca catgaatgcc
 481 aagatagccg atttcggatt atctaatatg atgtcagatg gtgaatttct gagaactagt
 541 tgtggatctc caaactatgc agcacctgaa gtcatctcag gcagattgta tgcaggtcct
 601 gaagttgata tctggagctg tggtgttatc ttgtatgctc ttctttgtgg caccctccca
 661 tttgatgatg agcatgtacc tacgttattt aagaagatcc gaggggggtgt cttttatatc
 721 ccagaatatc tcaatcgttc tgtcgccact ctcctgatgc atatgctgca ggttgaccca
 781 ctgaaacgag caactatcaa agacataaga gagcatgaat ggtttaaaca agatttgccc
 841 agttacttat ttcctgaaga cccttcctat gatgctaacg tcattgatga tgaggctgtg
 901 aaagaagtgt gtgaaaaatt tgaatgtaca gaatcagaag taatgaacag tttatatagt
 961 ggtgaccctc aagaccagct tgcagtggct tatcatctta tcattgacaa tcggagaata
1021 atgaaccaag ccagtgagtt ctacctcgcc tctagtcctc catctggttc ttttatggat
1081 gatagtgcca tgcatattcc cccaggcctg aaacctcatc cagaaaggat gccacctctt
1141 atagcagaca gccccaaagc aagatgtcca ttggatgcac tgaatacgac taagcccaaa
1201 tctttagctg tgaaaaaagc caagtggcat cttggaatcc gaagtcagag caaaccgtat
1261 gacattatgg ctgaagttta ccgagctatg aagcagctgg attttgaatg gaaggtagtg
1321 aatgcatacc atcttcgtgt aagaagaaaa aatccagtga ctggcaatta cgtgaaaatg
1381 agcttacaac tttacctggt tgataacagg agctatcttt tggactttaa aagcattgat
1441 gatgaagtag tggagcagag atctggttcc tcaacacctc agcgttcctg ttctgctgct
1501 ggcttacaca gaccaagatc aagttttgat tccacaactg cagagagcca ttcactttct
1561 ggctctctca ctggctcttt gaccggaagc acattgtctt cagtttcacc tcgcctgggc
1621 agtcacacca tggatttttt tgaaatgtgt gccagtctga ttactacttt agcccgttga
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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
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841 ctgcatgaga ctctggagac catcatcaac aggctagtgg aagcagaggt tcaccgactt
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Reviewed and approved by site quality representative.