

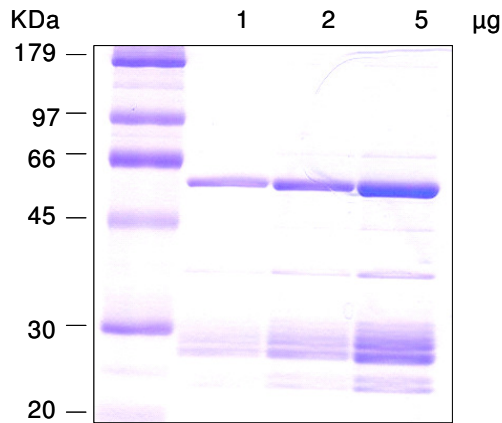
Certificate of Analysis

Product	AMPK alpha 1 (1-310), unactive human recombinant, expressed in E. coli, N-GST-fusion protein	
Cat No	PK-026-01	
Lot No	260907	
Description	Purified human recombinant AMPK alpha1 subunit (amino acids 1-310), expressed in E. coli. Suitable for activation by LKB1. Features a glutathione-S-transferase (GST) tag to facilitate removal of the enzyme from the reaction mixture. Purified by glutathione sepharose affinity chromatography. Sequence based calculated M.W. 63,260 Approved HUGO gene symbol: PRKAA1 Synonyms: AMPK-activated protein kinase, alpha 1 subunit, AMPKa1	
Quality	Protein concentration (Bradford with BSA as standard)	0.29 mg/ml
	Purity	> 60% by SDS PAGE
	Protease activity (Twining test)	none
Form	Liquid. In 50 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 0.05 % Brij, 10 % glycerol, pH 7.5	
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

SDS-PAGE analysis



Amino acid sequence information

M230 corresponds to M1 of BC037303, L539 corresponds to L310 of BC037303; the protein contains a thrombin cleavage site between the GST-tag and AMPK alpha 1

MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	50
EFPNLPYYID	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	100
DIRYGVSRIA	YSKDFETLKV	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	150
PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAI PQID	KYLKSSKYIA	200
WPLQGWQATF	GGGDHPPKSD	LVPRGSPNSM	ATAEKQKHDG	RVKIGHYILG	250
DTLGVGTFGK	VKVGKHELTG	HKVAVKILNR	QKIRSLDVVG	KIRREIQNLK	300
LFRHPHI IKL	YQVISTPSDI	FMVMEYVSGG	ELFDYICKNG	RLDEKESRRL	350
FQQILSGVDY	CHRHMVVHRD	LKPENVLLDA	HMNAKIADFG	LSNMMSDGEF	400
LRTSCGSPNY	AAPEVISGRL	YAGPEVDIWS	SGVILYALLC	GTLPFDDDHV	450
PTLFKKICDG	IFYTPQYLN	SVISLLKHML	QVDPMKRATI	KDIREHEWFK	500
QDLPKYLFPE	DPSYSSTMID	DEALKEVCEK	FECSEEEVLR	GRLERPHRD*	549

Activation of AMPK alpha 1 (1-310) by LKB1

Assay Components

Assay buffer 1 (AB1): 25 mM Tris-HCl, 1 mM DTT, pH 7.5

Protein kinase AMPK alpha 1, unactive

LKB1 (e.g. Upstate cat. # 14-596)

Magnesium/ATP Cocktail: 75 mM MgCl₂, 500 microM ATP

Assay Procedure

All compounds are pipetted into a microcentrigue tube on ice

1. 3.55 microliter AB1
2. 1 microliter LKB1 (0.1 mg/ml)
3. 3.45 microliter AMPK alpha 1 (0.29 mg/ml)
4. 2 microliter Magnesium/ATP Cocktail
6. Incubate 1 h at 30 °C.
7. Stop the reaction by setting samples on ice

AMPK *In Vitro* Kinase Assay

Assay Components

Assay buffer 2 (AB2): 20 mM Hepes, 0.4 mM DTT, 0.01 % Brij 35, pH 7.0

Substrate: AMPK substrate peptide HMRSAMSGHLVKRR, 400 microM

Protein kinase: AMPK activated by LKB1

Magnesium/ATP Cocktail: 75 mM MgCl₂, 500 microM ATP

Diluted [γ -³²P]ATP: Mix 197 microl Magnesium/ATP cocktail with 3 microliter (30 microCi) [γ -³²P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay Procedure

All compounds are pipetted into a microcentrigue tube on ice

1. Add 18 microliter AB2
2. Add 4 microliter SAMS peptide, 400 microM
3. Add 2 microliter AMPK alpha 1 from activation reaction (200 ng/assay)
4. Add 10 microliter of the diluted [γ -³²P]ATP
5. Incubate 10 min at 30 °C.
6. Stop the reaction by setting samples on ice
7. Remove 20 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager

Typically the activation of AMPK alpha 1 by LKB1 results in a specific activity of AMPK alpha 1 of > 150.000 pmol/mg/min

References

Hardie DG: AMPK-activated protein kinase as a drug target. *Annu Rev Pharmacol Toxicol* 2007, 47. 185-210.

Schimmak G, Defronzo RA, Musi N: AMPK-activated protein kinase: Role in metabolism and therapeutic implications. *Diabetes Obes Metab* 2006, 8, 591-602.

Marshall S: Role of insulin, adipocyte hormones, and nutrient sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity and cancer. *Sci STKE* 2006, 2006(341), re7