Recombinant AMPK Complex (A1+B2+G2) protein

Catalog No: 81405, 89405
Expressed In: Baculovirus

Quantity: 20, 1000 µg
Concentration: 0.4 µg/µl
Source: Human

Buffer Contents: Recombinant AMPK Complex (A1+B2+G2) Complex is supplied in 25 mM HEPES-NaOH pH 7.5, 300 mM NaCl, 10% glycerol, 0.04% Triton X-100 and 0.5 mM TCEP.

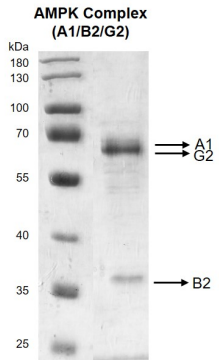
Background: AMPK is an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energyconsuming processes: inhibits protein, carbohydrate and lipid biosynthesis, as well as cell growth and proliferation. AMPK acts via direct phosphorylation of metabolic enzymes, and by longer-term effects via phosphorylation of transcription regulators. It also acts as a regulator of cellular polarity by remodeling the actin cytoskeleton; probably by indirectly activating myosin. AMPK regulates lipid synthesis by phosphorylating and inactivating lipid metabolic enzymes such as ACACA, ACACB, GYS1, HMGCR and LIPE and regulates fatty acid and cholesterol synthesis by phosphorylating acetylCoA carboxylase (ACACA and ACACB) and hormone-sensitive lipase (LIPE) enzymes, respectively. It also regulates insulin-signaling and glycolysis by phosphorylating IRS1, PFKFB2 and PFKFB3.

AMPK is a heterotrimer consisting of an alpha catalytic subunit (PRKAA1 or PRKAA2), and non-catalytic beta (PRKAB1 or PRKAB2) and gamma subunits (PRKAG1, PRKAG2 or PRKAG3). Beta non-catalytic subunit acts as a scaffold on which the AMPK complex assembles, via its C-terminus that bridges alpha and gamma subunits. Gamma non-catalytic subunit mediates binding to AMP, ADP and ATP, leading to activate or inhibit AMPK: AMP-binding results in allosteric activation of alpha catalytic subunit (PRKAA1 or PRKAA2) both by inducing phosphorylation and preventing dephosphorylation of catalytic subunits. ADP also stimulates phosphorylation, without stimulating already phosphorylated catalytic subunit. ATP promotes dephosphorylation of catalytic subunit, rendering the AMPK enzyme inactive.

Protein Details: Recombinant AMPK Complex (A1+B2+G2) that includes full length human PRKAA1 protein (accession number NP_996790.3) with a N-terminal FLAG tag and full length human PRKAB2 protein (accession number NP_005390.1) without tag and full length human PRKAG2 protein (accession number NP_057287.2) without tag was expressed in Hi5 cells. The molecular weights of PRKAA1, PRKAB2, PRKAG2 are 65.3 kDa, 30 kDa and 63.7 kDa,
respectively.

Application Notes: This product was manufactured as described in Protein Details. Where possible, Active Motif has developed functional or activity assays for recombinant proteins. Additional characterization such as enzyme kinetic activity assays, inhibitor screening or other biological activity assays may not have been performed for every product. All available data for a given product is shown on the lot-specific Technical Data Sheet.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.

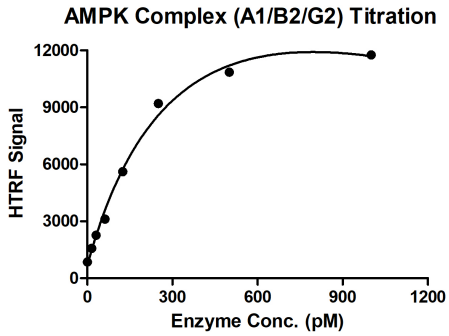


Recombinant AMPK Complex (A1+B2+G2) protein

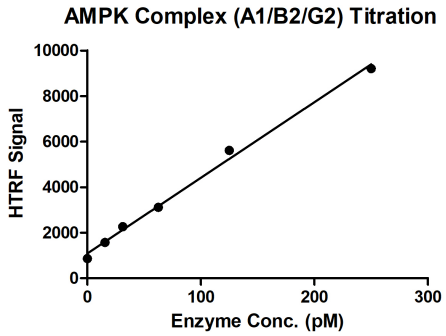
10% SDS-PAGE Coomassie staining

MW: PRKAA1: 65.3 kDa, PRKAB2: 30 kDa, PRKAG2: 63.7 kDa

Purity: ≥ 90%



HTRF assay for AMPK Complex (A1+B2+G2) activity
1 µM STK S1 substrate was incubated with different concentrations of AMPK Complex (A1+B2+G2) protein in a 10 µl reaction system containing 1×Enzymatic Buffer, 5 mM MgCl2, 1 mM DTT, 50 µM AMP and 100 µM ATP for 1 hr. The 10 µl detection reagents containing anti-STK antibody (1:100) and SA-XL665 (1:100) diluted with 1× Detection Buffer were added and incubated with the reactions for 30 min. All the operations and reactions were performed at room temperature. HTRF assay was used for detection.



HTRF assay for AMPK Complex (A1+B2+G2) activity
1 µM STK S1 substrate was incubated with different concentrations of AMPK Complex (A1+B2+G2) protein in a 10 µl reaction system containing 1×Enzymatic Buffer, 5 mM MgCl2, 1 mM DTT, 50 µM AMP and 100 µM ATP for 1 hr. The 10 µl detection reagents containing anti-STK antibody (1:100) and SA-XL665 (1:100) diluted with 1× Detection Buffer were added and incubated with the reactions for 30 min. All the operations and reactions were performed at room temperature. HTRF assay was used for detection.