AMPK (A1/B1/G3), His-tags Recombinant

Catalog: 40022

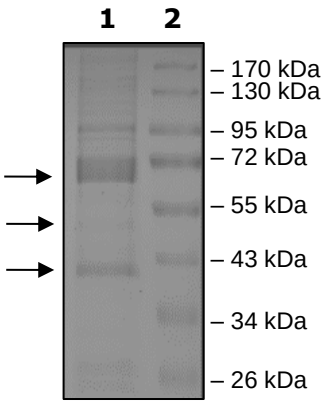
Lot: 230525

Product Information

Description:	Recombinant human AMPK (combination of A1/B1/G3 subunits) full length protein containing a C-terminal His-tag. This recombinant protein was affinity purified and is kinase active.
Species:	Human
Construct:	AMPK (A1/B1/G3-His)
Concentration:	0.1 mg/ml
Expression System:	Sf9
Purity:	70%
Format:	Aqueous buffer solution
Formulated In:	50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.25 mM DTT, 25% glycerol.
MW:	A1: 68 kDa; B1: 38 kDa; G3: 51 kDa
Genbank Accession:	A1: NM_006251; B1: NM_006253; G3: NM_017431
Stability:	At least 6 months at -80°C. Avoid freeze/thaw cycles.
Storage:	-80°C
Instructions for Use:	Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before opening. Aliquot into small volumes and flash freeze for long term storage. Avoid multiple freeze/thaw cycles.
Specific Activity:	162 pmol/min/µg
Assay Conditions:	Kinase activity was measured using ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the AMPK (A1/B1/G3) reaction. The ADP-Glo™ Reagent is added to terminate the reaction and quench the remaining ATP. The Kinase Detection Reagent is then added to convert ADP to ATP and to measure the newly converted ATP using a luciferase/luciferin reaction. Assay: In a 384 well plate, kinase activity was measured using SAMStide synthetic peptide substrate (HMRSAMSGLHLVKRR) diluted in 20 mM Tris-HCl, pH 7.5 to a final concentration of 1 mg/ml. Increasing amounts of AMPK (A1/B1/G3) kinase were incubated with a combined substrate in a final concentration of 0.3 mg/ml in 40 mM Tris-HCl, pH 7.4, 20 mM MgCl2, 0.1 mg/ml BSA, and 50 µM fresh DTT and a 0.5 mM AMP solution in 25 mM MOPs, pH 7.2. The reaction was initiated by adding a final concentration of 25 µM ATP and incubating for 40 minutes at room temperature. The reaction was then terminated by the addition of 5 µl of ADP-Glo™ Reagent followed by a spin and shake of the 384 well plate. A second incubation at room temperature for 40 minutes while shaking was performed. Luminescence was measured in the 384 well plate by the addition of 10 µl Kinase Detection Reagent followed by incubation at room temperature for an additional 30 minutes. The blank was determined from a “no kinase” sample.
Applications:	Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Quality Control Data

4-20% SDS-Page Coomassie Staining



Specific Activity