AMPK (A1/B1/G2), His-tag Recombinant

Catalog: 40021

Lot: 230525

Product Information

Description: Recombinant human AMPK (combination of A1/B1/G2 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/G2-His)

Concentration: 0.1 mg/ml

Expression System: Sf9 **Purity:** 70%

Format: Aqueous buffer solution

Formulated In: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM

DTT, 0.1 mM PMSF, 25% glycerol.

MW: A1: 68 kDa; B1: 38 kDa; G2: 65 kDa

Genbank Accession: A1: NM_006251; B1: NM_006253; G2: NM_001040633 **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: 780 pmol/min/μg

Assay Conditions: AMPK (A1/B1/G2) activity was measured by using a SAMStide synthetic peptide

substrate (HMRSAMSGLHLVKRR), diluted in distilled water to a final concentration of 1 mg/ml, in a [33P]-ATP based assay. Reaction was initiated by mixing increasing amounts of the AMPK (A1/B1/G2) with 1250 pmoles of [33P]-ATP in 5 mM MOPS, pH 7.2, 2.5 mM β -glycerol-phosphate, 5 mM MgCl₂, 0.4 mM EDTA, 50 ng/ μ l BSA prepared with 50 μ M DTT, 1 mM ATP and substrate at a final concentration of 200

μg/ml.

The reaction was initiated by addition of [33P]-ATP Assay Cocktail, followed by a 15-minute incubation at 30°C. The reaction was terminated by spotting the reaction mixture on phosphocellulose P81 paper, followed by three 10-minute washes with 1% phosphoric acid solution. Radioactivity was measured in a scintillation counter. The corrected activity (RLU) was calculated by removing the blank value for each sample. The Kinase Specific Activity was calculated as follows: RLU / [(specific activity of [33P]-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)] * [(Reaction Volume) / (Spot Volume)]. The blank was determined from a "no substrate" sample by replacing the substrate solution with an equal volume of

distilled water.

Applications: Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

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