Lot: 221018

## **Product Information**

**Construct**: PKAcβ (GST-Full Length)

Concentration: 0.10 mg/ml Species: Human

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

Expression System: Sf9

Format: Aqueous buffer solution

Stability: At least 6 months at -80°C. Avoid freeze/thaw cycles.

Storage: -80°C
Genbank Accession: NM 002731

MW: 65 kDa
Purity: 90%

Specific Activity: 460 pmol/min/μg

Assay Conditions: Kinase activity was measured using the ADP-Glo™ Kinase Assay Kit (Promega; Cat#

V9101) which quantifies the amount of ADP produced. The ADP-Glo™ Reagent is added to terminate the reaction and deplete the remaining ATP. The Kinase Detection Reagent is then added to convert ADP to ATP and to measure the newly

synthesized ATP using a luciferase reaction.

PKAcβ kinase activity was measured by using CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled water to a final concentration of 1 mg/ml. Reaction was initiated by mixing increasing amounts of the PKAcβ with 25  $\mu$ M ATP in 40 mM Tris-HCl, pH 7.4, 20 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA prepared with 250  $\mu$ M DTT and

20 μg/ml substrate.

After a 40-minute incubation at 37°C, the reaction was terminated by addition of the AMP-Glo™ Reagent followed by a subsequent 40-minute incubation at room temperature. Kinase Detection Reagent was then added and incubated for another 30 minutes. Detection of luminescence was measured using the Luminescence Module Protocol on GloMax®-Multi Microplate reader. The corrected activity (RLU) was calculated by removing the blank value for each sample divided by the (specific activity of ADP in RLU/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg). The blank was determined from a "no kinase" sample by replacing the enzyme

working solution with an equal volume of Kinase Dilution Buffer IX (1X).

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



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