CAMK1δ Active human, recombinant GST-tagged, expressed in Sf9 cells

Catalog Number C9619
Lot Number 019K1566
Storage Temperature –70 °C

Synonyms: RP11-462F15.1; CKLiK; CaM-K1; CaMKID

Product Description
CAMK1δ or Ca\(^{2+}\)/calmodulin-dependent kinase I-like kinase (CKLiK) is activated by Ca\(^{2+}\) and calmodulin, and is detected in CD34+-derived neutrophils and eosinophils, as well as in mature peripheral blood granulocytes.\(^1\) CAMK1δ exhibits Ca\(^{2+}\)/CaM-dependent activity that is enhanced \textit{in vitro} by phosphorylation of Thr\(^{180}\) by CaM-K kinase (CaM-KK)alpha, consistent with detection of CAMK1δ-activating activity in HeLa cells.\(^2\)

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 153498. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~68 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 117–159 nmole/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl\(_2\), 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.
Kinase Solution – Dilute the Active CAMK1\(\delta\) (0.1 \(\mu\)g/\(\mu\)l) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active CAMK1\(\delta\) kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 \(\mu\)l aliquots at –20 °C.

\(\gamma\)-\(\beta\)-\(32\)P-ATP Assay Cocktail (250 \(\mu\)M) – Combine 5.75 ml of Kinase Assay Buffer, 150 \(\mu\)l of 10 mM ATP Stock Solution, 100 \(\mu\)l of \(\gamma\)-\(32\)P-ATP (1 mCi/100 \(\mu\)l). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (KKALRRQETVDAL-amide) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

**Kinase Assay**
This assay involves the use of the \(32\)P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active CAMK1\(\delta\), Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The \(\gamma\)-\(32\)P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 \(\mu\)l:
   - 10 \(\mu\)l of Kinase Solution
   - 7.5 \(\mu\)l of Substrate Solution
   - 2.5 \(\mu\)l of 5 mM CaCl\(_2\) solution containing 0.75 \(\mu\)g Calmodulin
3. Set up a blank control as outlined in step 2, substituting 7.5 \(\mu\)l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 \(\mu\)l of the \(\gamma\)-\(32\)P-ATP Assay Cocktail, bringing the final reaction volume to 25 \(\mu\)l. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 \(\mu\)l of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of –10 minutes each.
7. Set up a radioactive control to measure the total \(\gamma\)-\(32\)P-ATP counts introduced into the reaction. Spot 5 \(\mu\)l of the \(\gamma\)-\(32\)P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

**Calculations:**
1. Specific Radioactivity (SR) of ATP (cpm/nmole)
   
   \[ SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}32\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}} \]
   
   cpm – value from control (step 7)
   
   nmole – 1.25 nmole (5 \(\mu\)l of 250 \(\mu\)M ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)
   
   \[ \text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{\text{SR} \times E \times T} \]
   
   \(\Delta \text{cpm} = \text{cpm of the sample} – \text{cpm of the blank (step 3)}\)
   
   25 = total reaction volume
   
   20 = spot volume
   
   T = reaction time (minutes)
   
   E = amount of enzyme (mg)

**References**

TLD, MAM 02/09-1