

## Product Information

**CAMK2 $\beta$ , Active**  
**human, recombinant**  
**GST-tagged, expressed in Sf9 cells**

Catalog Number **C9994**  
Lot Number 069K0832  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

Synonyms: CAMKB; CAM2; CAMK2; MGC29528

### Product Description

CAMK2 $\beta$  belongs to the serine/threonine protein kinase family and to the type II multifunctional  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase subfamily. CAMK2 $\beta$  showed wide tissue and cell distribution, and one of CAMK2 $\beta$  variant predominated in adult brain.<sup>1</sup> The ratio of CAMK2 $\alpha$  and CAMK2 $\beta$  protein levels were inversely related during activity in hippocampal neurons.<sup>2</sup> CAMK2 $\beta$  is a prominent kinase in the central nervous system and may function in long-term potentiation and neurotransmitter release.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 172081. It is supplied in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.2 mM DTT, and 25% glycerol.

Molecular mass: ~58 kDa

Purity:  $\geq 70\%$  (SDS-PAGE, see Figure 1)

Specific Activity: 4,820–6,521 nmole/min/mg  
(see Figure 2)

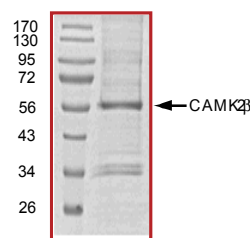
### Precautions and Disclaimer

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.

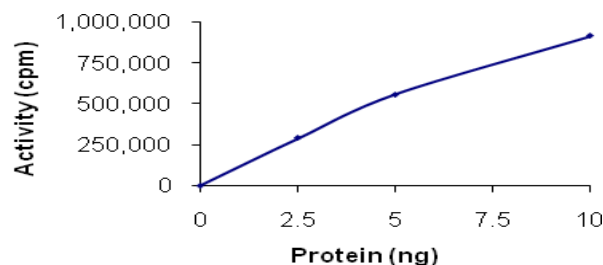
### Storage/Stability

The product ships on dry ice and storage at  $-70\text{ }^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Lot Number 069K0832:  
>75% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number 069K0832:  
5,670 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM  $\text{MgCl}_2$ , 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/ $\mu\text{l}$  BSA solution.

Kinase Solution – Dilute the Active CAMK2 $\beta$  (0.1  $\mu\text{g}/\mu\text{l}$ ) 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 CAMK2 $\beta$  kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200  $\mu\text{l}$  aliquots at  $-20\text{ }^{\circ}\text{C}$ .

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

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  $^{32}\text{P}$  radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active CAMK2 $\beta$ , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The  $\gamma$ - $^{32}\text{P}$ -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  $\mu\text{l}$ :
  - 10  $\mu\text{l}$  of Kinase Solution
  - 7.5  $\mu\text{l}$  of Substrate Solution
  - 2.5  $\mu\text{l}$  of 5 mM  $\text{CaCl}_2$  solution containing 0.75  $\mu\text{g}$  Calmodulin
3. Set up a blank control as outlined in step 2, substituting 7.5  $\mu\text{l}$  of cold water ( $4\text{ }^{\circ}\text{C}$ ) for the Substrate Solution.
4. Initiate each reaction with the addition of 5  $\mu\text{l}$  of the  $\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail, bringing the final reaction volume to 25  $\mu\text{l}$ . Incubate the mixture in a water bath at  $30\text{ }^{\circ}\text{C}$  for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20  $\mu\text{l}$  of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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  $\sim 10$  minutes each.
7. Set up a radioactive control to measure the total  $\gamma$ - $^{32}\text{P}$ -ATP counts introduced into the reaction. Spot 5  $\mu\text{l}$  of the  $\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail on a pre-cut 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)

$$\text{SR} = \frac{\text{cpm of } 5\ \mu\text{l of } \gamma\text{-}^{32}\text{P}\text{-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)  
nmole – 1.25 nmole (5  $\mu\text{l}$  of 250  $\mu\text{M}$  ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{\text{SR} \times \text{E} \times \text{T}}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)  
 $\Delta\text{cpm}$  = cpm of the sample – cpm of the blank (step 3)  
25 = total reaction volume  
20 = spot volume  
T = reaction time (minutes)  
E = amount of enzyme (mg)

#### References

1. Tombes, R.M. et al., Identification of novel human tumor cell-specific CaMK-II variants. *Biochim. Biophys. Acta*, **1355**, 281-292 (1997).
2. Thiagarajan, T.C. et al., Alpha- and beta-CaMKII: inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron*, **36**, 1103-1114 (2002).

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