

Saint Louis, Missouri 63103 USA Telephone (800) 325-5832 (314) 771-5765 Fax (314) 286-7828 email: techserv@sial.com sigma-aldrich.com

# ProductInformation

ANTI- CAM KINASE KINASE a (CAMKKa) Developed in Rabbit Affinity isolated Antibody

Product Number C7099

## **Product Description**

Anti-CaM Kinase Kinase  $\alpha$  (CaMKK $\alpha$ ) is developed in rabbit using a synthetic peptide (GEGGKSPELPGVQ-EDEAAS) corresponding to the C-terminal region of rat CaMKK $\alpha$  (amino acids 487-505) conjugated to KLH as immunogen. This sequence has no homology to rat CaMKK $\beta$  and human CaMKK $\alpha$  and CaMKK $\beta$  isoforms. Anti-CaMKK $\alpha$  is affinity-purified using the immunogenic peptide immobilized on agarose.

Anti-CaM Kinase Kinase  $\alpha$  (CaMKK $\alpha$ ) recognizes rat CaMKK $\alpha$  (~66 kD). Applications include the detection and localization of CaMKK $\alpha$  (~66 kD) by immunoblotting and immunohistochemistry. Staining of CaMKK $\alpha$  in immunoblotting is specifically inhibited with CaMKK $\alpha$ immunizing peptide (CaMKK $\alpha$ , rat, amino acids 487-505).

Ca<sup>2+</sup>/Calmodulin dependent protein kinases (CaM Kinase I, II and IV) are considered to play a central role in many cellular functions in response to Ca<sup>2+</sup> signaling, including synthesis and secretion of neurotransmitters, axonal transport, long term potentiation (LTP), receptor function, modification of the cytoskeleton and regulation of gene expression.<sup>1,2</sup> Ca<sup>2+</sup>/Calmodulin dependent protein Kinase Kinase (CaMKK) <sup>3,4</sup> consists of two distinct Ser/Thr protein kinases, CaMKKa and CaMKKB (66-68kD), <sup>5-9</sup> which phosphorylate and activate CaMKI and CaMKIV as part of a unique  $Ca^{2+}/CaM$ -regulated kinase cascade.<sup>2,4,8,9</sup> Activation occurs through selective phosphorylation of an equivalently positioned Thr in the activation T-loop of CaMKI and CaMKIV, Thr<sup>111</sup> and Thr<sup>196</sup>/Thr<sup>200</sup>, respectively. CaMKK $\alpha$  and CamKK $\beta$ isoforms share considerable sequence homology with each other (65% identity and 80% similarity). CaMKK $\alpha$ and CamKK $\beta$  are both abundantly expressed in the brain, and are thought to be upstream regulators of neuronal Ca<sup>2+</sup>/CaM-dependent processes. CaMKKβ is also expressed at lower levels, in other tissues, (e.g. thymus, testis and spleen), whereas CaMKKα appears to be more restricted to the brain.

CaMKK $\alpha$  and CaMKK $\beta$  show differential regional and subcellular localizations in different tissues (e.g. in the rat brain).<sup>6,7,10</sup> The distribution of CaMKK $\alpha$  has been shown to correlate with that of CaMKI, and the distribution of CaMKK $\beta$  correlates with CaMKIV. It has been suggested that CaMKK $\alpha$  and CaMKK $\beta$  may have an important role in the regulation of CaMKI and CaMKIV activities, respectively. Activation of the PKB pathway by CaMKK appears to be important in protection of neurons from programmed cell death during development.<sup>11</sup>

## Reagents

Anti-CaM Kinase Kinase  $\alpha$  (CaMKK $\alpha$ ) is supplied as affinity isolated antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide (see MSDS)\* as a preservative.

Protein concentration is approximately 1 mg/ml by absorbance at 280 nm.

#### **Precautions and Disclaimer**

\* Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

## Storage/Stability

For continuous use, store at 2-8°C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

### **Product Profile**

A minimum working dilution of 1:5,000 is determined by immunoblotting using a rat brain extract.

A minimum working dilution of 1:500 is determined by immunohistochemistry using formalin-fixed, paraffinembedded sections of rat brain.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

#### References

1. Hanson, P.I. and Schulman, H., Ann. Rev. Biochem. **61**, 559 (1992).

- 2. Soderling, T.R., Trends Biochem. Sci. **24** 232 (1999).
- 3. Okuno, S. et al., J. Biochem. **116**, 923 (1994).
- 4. Tokumitsu, T., et al., J. Biol. Chem. **270**, 19320 (1995).
- 5. Edelman, A.M., et al., J. Biol. Chem. **271**, 10806 (1996).
- 6. Okuno, S. et al., J. Biochem. 119, 1176 (1996).
- Anderson, K.A., et al., J. Biol. Chem. 273, 31880 (1998).
- Matsushita, M., et al., J. Biol. Chem. 273, 21473 (1998).
- Park, I-K., and Soderling, T.R., J. Biol. Chem. 270, 30464 (1995).
- 10. Nakamura, Y., et al., Neurosci, Lett. 204 61 (1996).
- 11. Yano, S., et al., Nature **396** 584 (1998).

lpg 10/99

Sigma brand products are sold through Sigma-Aldrich, Inc.