Affinity Purified Rabbit Anti-Phospho-CaM Kinase II (T286)
Certificate of Analysis

ORDERING INFORMATION

Catalog Number: PPS002

Lot Number: 1466786

Size: 100 µL (sufficient for 10 mini blots)

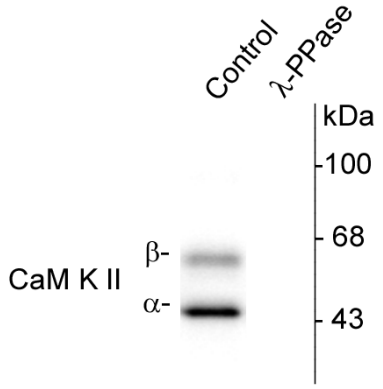
Storage: ≤ -20° C

Specificity: Human, mouse, rat, bovine, chicken, *Xenopus* for the ~50 kDa α-CaM Kinase II phosphorylated at T286

Immunogen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-α-CaM Kinase II (T286)

Ig Type: rabbit IgG

Applications: Western blot



Western Blot of rat brain lysate showing specific immunolabeling of the ~50 kDa α-CaMKII subunit phosphorylated at T286 and the ~60 kDa β-CaMKII subunit phosphorylated at T287 (Control). The phosphospecificity of this labeling is demonstrated by treatment with 1200 U of λ Phosphatase (λ-PPase) for 30 minutes before being exposed to the anti-phospho-CaMKII (T286). The immunolabeling is completely eliminated by treatment with λ-PPase.

Description
Calmodulin Kinase II (CaMKII) is a 500 kDa, 8 - 12 subunit multimer that belongs to the Ser/Thr protein kinase family. It is ubiquitously expressed and interacts with a very diverse group of substrates. In rat, there are four possible subunits/isozymes (α, β, γ, δ) that vary from 480 - 540 amino acids in length. The α- and β-isozymes predominate in the brain. Each subunit contains a catalytic, autoregulatory, and subunit-association domain. The enzyme complex is inactive, due to the association of an internal pseudosubstrate motif with each subunit's catalytic domain. CaMKII is regulated by calmodulin (CaM), an intracellular receptor for calcium. Following an influx of calcium, two Ca^{++}-CaM complexes interact with inactive CaMKII at the autoregulatory site of two adjacent CaMKII subunits. This dissociates the catalytic site from the pseudosubstrate motif, allowing for the auto(cross)-phosphorylation of T286 on one α-subunit (T287 on a β-subunit) by the catalytic site on an adjacent subunit. The T286 phosphorylation event blocks a reassociation of the catalytic domain with the internal pseudosubstrate motif, resulting in prolonged activation. Once activated, an autoinhibitory program ensues. The dissociation of Ca^{++}-CaM from CaMKII exposes a Thr at position 305. CaMKII autophosphorylation of Thr at this site downregulates existing CaMKII activity.

Preparation
Prepared from rabbit serum by affinity purification via sequential chromatography on phospho- and dephospho-peptide affinity columns.

Formulation
100 µL in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/mL BSA and 50% glycerol.

Storage
For long-term storage, ≤ -20° C is recommended. Product is stable at ≤ -20° C for at least 1 year.

Specificity
Specific for the ~50 kDa α-CaM Kinase II phosphorylated at T286 and the ~60 kDa β-CaM Kinase II phosphorylated at T287 in Western blots of rat brain lysates.

Applications
Western blot - 1:1000

Optimal dilutions should be determined by each laboratory for each application.

References

1. Griffith, L.C. (2004) J. Neurosci. **24**:8391.
2. Hudmon, A. and H. Schulman (2002) Annu. Rev. Biochem. **71**:473.
3. Lin, C.R. *et al*. (1987) Proc. Natl. Acad. Sci. USA **84**:5962.
4. Thiel, G. *et al*. (1988) Proc. Natl. Acad. Sci. USA **85**:6337.
5. Elgersma, Y. *et al*. (2002) Neuron **36**:493.

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