

Product Information

Anti-phospho-CaM Kinase II (CaMKII) [pThr³⁰⁵]

produced in rabbit, whole antiserum

Catalog Number **P5997**

Product Description

Anti-phospho-CaM Kinase II [pThr³⁰⁵] is produced in rabbit using a synthetic peptide corresponding to amino acid residues surrounding the rat brain CaM Kinase II phosphorylated on threonine 305 as immunogen. The sequence is conserved in human, mouse, chicken, bovine and rat (100% homology).

The antibody recognizes rat CaM Kinase II α (50 kDa) and β (60 kDa) phosphorylated at threonine 305. Due to the high degree of homology, the antibody is expected to crossreact with mouse, bovine, human, chicken and *Xenopus*. It has been used in immunoblotting and dot blot applications.

Calcium/Calmodulin dependent protein kinase II (CaMKII) belongs to the family of Ser/Thr protein kinases including CaMKI and CaMKIV. CaMKII is involved in many cellular functions in response to calcium signaling, including synthesis and secretion of neurotransmitters, axonal transport, long term potentiation (LTP) and spatial learning, receptor function and regulation of gene expression. CaM kinase II is one of the most abundant protein kinases in the mammalian brain, with the highest expression in neurons of the hippocampus (~2% of total protein) and the cerebral cortex, where it plays a critical role in LTP, a cellular model of learning and memory. CaMKII consists of a family of four related isoforms CaMKII α , β , γ and δ (50-60kDa). The CaMKII α and β isoforms are predominantly expressed in the brain, localized mainly in the cytosol and postsynaptic densities (PSDs), whereas the CaMKII γ and δ isoforms are expressed in all tissues. CaMKII contains a catalytic and regulatory domain. The regulatory domain consists of an autoinhibitory and calmodulin binding site. CaMKII forms multimers of 8-12 subunits composed primarily of the α and β subunits.

In the CNS, postsynaptic Ca²⁺ influx triggers rapid autophosphorylation and stable activation of CaMKII in a Calcium/calmodulin dependent manner at a threonine residue in the autoinhibitory domain (threonine 296 in

CaMKII α and threonine 287 in CaMKII β). Autophosphorylation of CaMKII α at threonine 296 has been shown to be required for LTP and learning. CaMKII activation results in switching of the kinase to a Ca²⁺/CaM- independent state and its translocation to the PSD. PSD-associated CaMKII in turn phosphorylates ionotropic glutamate receptors (e.g., NMDAR, AMPA-R), thus providing a mechanism for increased synaptic signaling during LTP. Autophosphorylation of Thr³⁰⁵ inhibits the activity of CaM Kinase II. Phosphorylation at this site appears to reduce the association of CaM kinase II with the PSD and reduce LTP and learning.

Reagent

The antibody is provided as whole rabbit antiserum.

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

Store at -20 °C. For extended storage, freeze in working aliquots. 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.

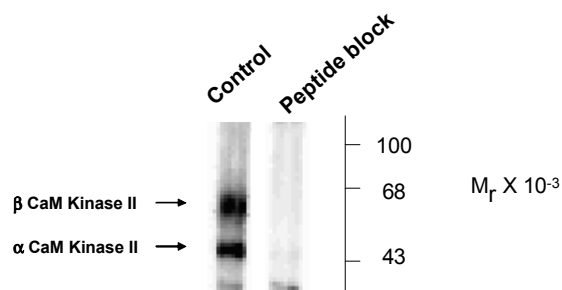
Product Profile

The supplied reagent is sufficient for 10 blots. A minimum working dilution of 1:1,000 is determined by immunoblotting using a rat brain lysates.

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

Results

Anti-Phospho Thr³⁰⁵ CaM Kinase II



The Figure 1 shows the results of immunoblot and peptide blocking.

1. 10 μ g of rat brain containing CaMKII α and β was lysed and applied to immunoblot.
2. Anti-phospho-(CaMKII) [pThr³⁰⁵] was applied at 1:1000 dilution.
3. The antibody produced bands with both isoforms.
4. The phosphorylated peptide used as immunogen specifically blocked both isoforms, whereas corresponding non-phosphorylated peptide did not block the immunolabeling (not shown).

References

1. Elgersma, Y, et al., Inhibitory Autophosphorylation of CaMKII Controls PSD Association, Plasticity, and Learning, *Neuron*, **36**, 493–505 (2002).
2. Kennedy, M. B., Signal-processing machines at the postsynaptic density, *Science*, **290**, 750–754 (2000).
3. Shen, K. and Meyer, T. Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation., *Science*, **284**, 162-166 (1999).
4. Leonard, A.S., et al., Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. *Proc. Natl. Acad. Sci. USA*, **96**, 3239-3244 (1999).
5. Derkach, V., et al., Ca²⁺/calmodulin-kinase II enhances channel conductance of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors., *Proc. Natl. Acad. Sci. USA*, **96**, 3269-3274 (1999).

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