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A010-50 anti-CaMKII phospho-Thr286 antibody 20 μ l

Background: Calcium/calmodulin-dependent protein kinase II (CaMKII) is a ubiquitous, multifunctional serine/threonine kinase involved in various calcium regulated cellular functions (Schulman and Braun, 1999). The multimeric protein is composed of 8 -12 individual protein kinase subunits, which are the products of four separate genes, α , β , γ , δ , and are expressed in a tissue-specific manner (Schulman and Braun, 1999). Subunit phosphorylation at Thr-286 (or 287 depending on isoform) results in an autonomous Ca²⁺/CaM independent activity and in an increased Ca²⁺/CaM binding affinity. This allows for CaMKII to retain maximal catalytic activity for prolonged times at low Ca²⁺ concentrations and partial activity in the absence of bound Ca²⁺/CaM (Braun and Schulman, 1995).

Description: Lyophilised rabbit polyclonal serum containing IgG antibody specific to all isoforms of CaMKII phospho-Thr286 (or Thr287 in β, δ, γ isoforms of CaMKII).

Immunogen: Synthetic peptide (M₂₈₁HRQET(PO₃H₂)VDC₂₈₉), corresponding to amino acids surrounding the phosphorylated threonine residue at position 286 of CaMKII (α isoform or Thr287 of other isoforms), conjugated to KLH.

Specificity and Species Cross Reactivity: Specific for CaMKII phospho-Thr286 (or 287) α , β , γ and δ isoforms in rat, human and mouse. Data below demonstrate recognition of α and β isoforms which differ in sequence at one position.

Applications: ELISA, Western blot 1:5000 dilution.

Storage: Store antibody desiccated at 4C when dry, and frozen (-20C or -80C) in small aliquots when reconstituted with 20 μ l deionised water.

Epitope sequence alignment CaMKII α (281-289)

Epitope	MHRQETVDC	Epitope	MHRQETVDC
Alpha/delta	MHRQETVDC	Beta/gamma (282-290)	
Human	MHRQETVDC	Human	MHRQETV[E]C ₂₉₀
Chicken	MHRQETVDC	Chicken	MHRQETV[E]C
Chimpanzee	MHRQETVDC	Chimpanzee	MHRQETV[E]C
Cow	MHRQETVDC	Cow	MHRQETV[E]C
Horse	MHRQETVDC	Horse	MHRQETV[E]C
Mouse	MHRQETVDC	Mouse	MHRQETV[E]C
Opossum	MHRQETVDC	Opossum	MHRQETV[E]C
pig	MHRQETVDC	pig	MHRQETV[E]C
Platypus	MHRQETVDC	Platypus	MHRQETV[E]C
Purple sea urchin	MHRQETVDC	Purple sea urchin	MHRQETV[E]C
rabbit	MHRQETVDC	rabbit	MHRQETV[E]C
Rat	MHRQETVDC	Rat	MHRQETV[E]C
Rhesus monkey	MHRQETVDC	Rhesus monkey	MHRQETV[E]C
sheep	MHRQETVDC	sheep	MHRQETV[E]C
Xenopus	MHRQETVDC	Xenopus	MHRQETV[E]C

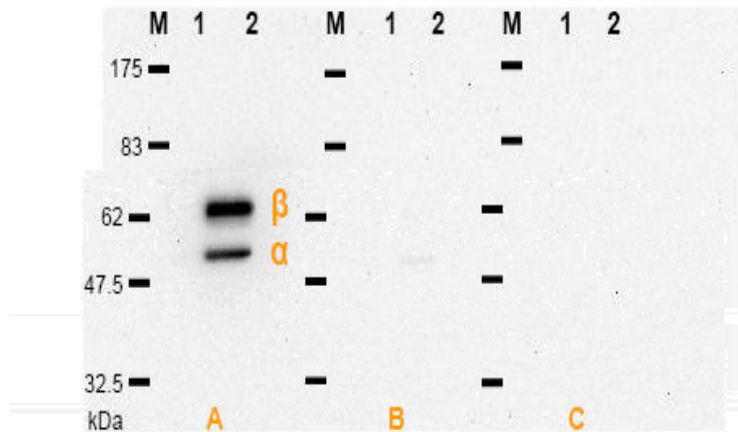


Figure 1: Detection of CaMKII phosphorylated at Thr-286 in rat brain lysate 50μg rat brain homogenate was phosphorylated by Ca²⁺-CaM and MgATP addition (30C, 1min: lane 2) or incubated without ATP (lane 1). Proteins were electrophoresed using 12% SDS-PAGE gels and transferred to PVDF. Thr-286 (or 287 β-isoform) phosphorylation was detected using 1:5000 dilution of anti-CaMKII phospho-Thr286 (A010-50) and peroxidase based chemiluminescence detection (Panel A). The α and β isoforms of CaMKII are both detected with this antibody. All staining was specific as staining was inhibited by processing identical blots in parallel with antibody A010-50 (1:5000) plus 1μM (panel B) or 10μM (panel C) epitope peptide (P010-50).

References:

Braun, A.P., & Schulman, H. 1995) *Ann. Rev. Physiol.* **57**, 417-445
 Schulman, H. & Braun, A.P. (1999) in *Calcium as a Cellular Regulator*, E. Carafoli & C.B. Klee (Eds.), pp 311-343, Oxford University Press, New York