

CaMKII (pThr286 or 287) pAb serum

Quality Control Certificate of Analysis

Catalogue No.: A010-50

Unit Size: 20 µl

Lot No: 0412-05

Background: Ca²⁺/calmodulin-dependent kinase II (CaMKII) is a ubiquitous, multifunctional serine/threonine kinase involved in translating Ca²⁺- transient signals into cellular responses (Shulman & Braun, 1999). Four separate CaMKII genes are expressed in man (α, β, δ, γ) with isoforms within each gene. CaMKII δ and γ are abundant in the heart. CaMKII is activated by Ca²⁺/CaM binding, and becomes activated for prolonged periods of time following autophosphorylation of Thr-286 or Thr-287 (equivalent residues in different isoforms). This antibody recognizes the active, autophosphorylated enzyme (all isoforms) and thus can measure the abundance of active CaMKII. Prolonged activation of CaMKII is associated with a variety of vascular and cardiac diseases including stroke (Vest et al., 2010), atrial fibrillation (Dobrev & Wehrens, 2010) cardiac arrhythmia & sudden death (van Oort et al., 2010), cardiac apoptosis (Wang et al., 2004), and heart failure (Erickson et al., 2011).

Description: Lyophilised **Rabbit** polyclonal anti-serum to CaMKII Phospho Thr-286/287 (A010-50).

Immunogen: Synthetic peptide (M₂₈₁HRQET(PO₃H₂)VDC₂₈₉) corresponding to amino acids surrounding the phosphorylated threonine residue at position 286 (or 287) of CaMKII, which was conjugated to keyhole limpet hemocyanin (KLH) by carbodiimide cross linkage.

Antibody Isotype: IgG.

Antibody Purity: Raw Serum.

Specificity: The antibody recognises both of the α and β subunits of CaMKII when phosphorylated at Thr-286. Binding of the antibody to its target epitope is blocked in the presence of a phosphopeptide containing the CaMKII Phospho Thr-286 epitope.

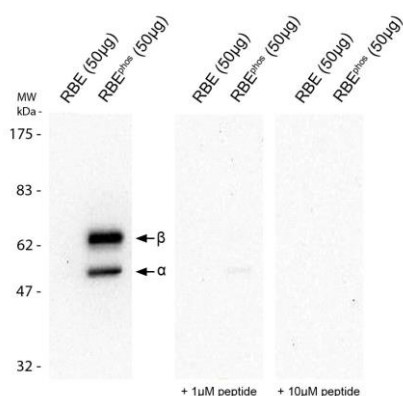
Species Cross Reactivity: Reacts with rat. Not yet tested in other species. **However, from primary sequence analysis, the antibody will recognise Phospho Thr-286 (or 287) in all isoforms (α, β, γ and δ) from human, rat and mouse.** The antibody is also expected to react with various isoforms from chicken, cow, pig, rabbit, zebrafish, and *Xenopus laevis*

Vial Constituents: Lyophilised A010-50 Rabbit anti-serum (20 µl)

Storage Instructions: Lyophilised antibody is stable at 4°C when stored with desiccant. Reconstitute lyophilised powder in 20 µl of 18 MΩ H₂O, aliquot and store frozen at -80°C for 1 year. Avoid freeze - thaw cycles.

Tested Applications: **WB 1:5000.** Not yet tested in other applications, therefore, optimal dilutions/concentrations should be determined by the user.

Epitope	PO ₃ H ₂ Specific										
	280									290	
Human	-	M	H	R	Q	E	T	V	D	C	-
Mouse	C	M	H	R	Q	E	T	V	D	C	L
Rat	M	M	H	R	Q	E	T	V	E	C	L
Xenopus	C	M	H	R	Q	E	T	V	D	C	L
Drosophila	V	V	H	R	Q	E	T	V	D	C	L



A010-50 (1:5000)

1 µl in 5 ml of Tris buffer + 5% milk powder 16 hours at 4°C

Secondary antibody = Goat anti Rabbit HRP
Chemiluminescent detection

Lane 1: 50 µg of Rat Brain Extract incubated with Ca²⁺ + Calmodulin
Lane 2: 50 µg of Rat Brain Extract incubated with Ca²⁺ + Calmodulin + ATP
Lane 3+4: Identical to lanes 1+2
Lane 5+6: Identical to lanes 1+2

WB using 1:5000 CaMKII Phospho threonine-286 anti-serum (A010-50) against 50µg of Rat Brain Extract incubated for 1 minute at 30°C with Ca²⁺, Calmodulin +/- MgATP. CaMKII anti-serum detected α and β kinase subunits in brain samples incubated with ATP. The staining was inhibited in identical A010-50 blots through the addition of 1µM and 10µM immunising peptide. 12% SDS-PAGE gel. PVDF membrane.

Related Products:

CaMKII Phospho Thr-286 epitope peptide (P010-50), CaMKII Phospho Thr-286 Affinity Purified Antibody (A010-50AP)

Background References:

- Dobrev, D., & Wehrens, X.H.T. (2010) Trends in Cardio. Medicine 20, 30-34
- Erickson, J.R., He, B.J., Grumbach, I.M., & Anderson, M.E. (2011) CaMKII in the cardiovascular system: sensing redox states. Physiol. Rev. 91, 889-915.
- Van Oort, R.J. et al. (2010) Circulation 122, 2669-79
- Shulman, H. & Braun, A.P. (1999) in Carafoli E. & Klee, C. (Eds.) Calcium as a Cellular Regulator, Oxford University Press.
- Vest, R. S., O'Leary, H., Coltrap, S.J., Kindy, M.S., & Bayer, K.L. (2010) Effective post-insult neuroprotection by a novel Ca²⁺/Calmodulin-dependent protein kinase II (CaMKII) inhibitor. J. Biol. Chem. 285, 20675-82
- Wang, W., Zhu, W., Wang, S., Yang, D., Crow, M.T., Xiao, R-P. & Cheng, H. (2004) Sustained β1-adrenergic stimulation modulates cardiac contractility by Ca²⁺/calmodulin kinase signaling pathway. Circ. Res. 95, 798-806.