

## CaMKII (pThr286 or 287) pAb serum

### Quality Control Certificate of Analysis

Catalogue No.: A010-50AP

Unit Size: 50 µl

Lot No.: 0611-04

**Background:** Ca<sup>2+</sup>/calmodulin-dependent kinase II (CaMKII) is a ubiquitous, multifunctional serine/threonine kinase involved in translating Ca<sup>2+</sup>-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 Ca<sup>2+</sup>/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:** Affinity Purified **Rabbit** polyclonal antibody to CaMKII Phospho Threonine-286/287 (A010-50AP)

**Immunogen:** Synthetic peptide (M<sub>281</sub>HRQET(PO<sub>3</sub>H<sub>2</sub>)VDC<sub>289</sub>) 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:** Protein A affinity purified.

**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.

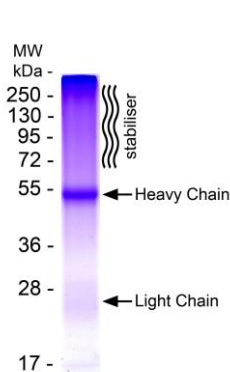
**Species Cross Reactivity:** Reacts with rat. Not yet tested in other species. **However, from primary sequence analysis, the antibody will recognise Phospho Thr-286 (or 287) in all isoforms (α, β, γ and δ) from human, rat and mouse.** The antibody is also expected to react with various isoforms from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

**Vial Constituents:** Lyophilised affinity purified A010-50AP Ab (50 µl) in 0.1M Tris-citrate pH 7.4 with 20%v/v stabiliser solution.

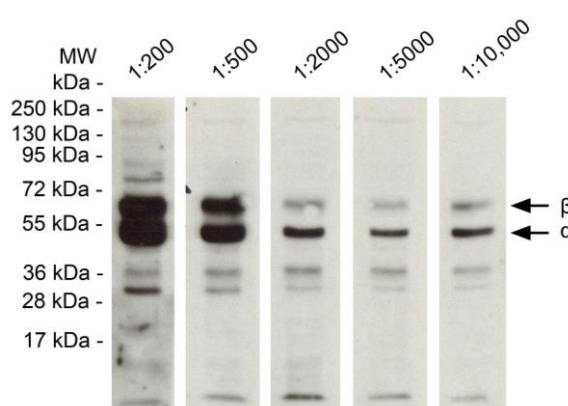
**Storage Instructions:** Lyophilised antibody is stable at 4°C when stored with desiccant. Reconstitute lyophilised powder in 50 µl of 18 MΩ H<sub>2</sub>O, aliquot and store frozen at -80°C for 1 year. Avoid freeze - thaw cycles.

**Tested Applications:** **WB 1:1000.** Not yet tested in other applications, therefore, optimal dilutions/concentrations should be determined by the user.

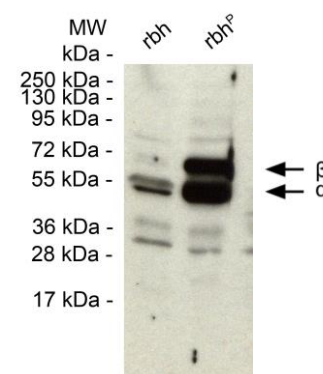
Epitope	PO <sub>3</sub> H <sub>2</sub> Specific										
	280									290	
Human	-	M	H	R	Q	E	T	V	D	C	-
Mouse	C	M	H	R	Q	E	T	V	D	C	L
Rat	M	M	H	R	Q	E	T	V	E	C	L
Xenopus	C	M	H	R	Q	E	T	V	D	C	L
Drosophila	V	V	H	R	Q	E	T	V	D	C	L



12% SDS-PAGE gel showing 10 µg of antibody A010-50AP



50 µg of phosphorylated rat brain homogenate probed with A010-50AP (1:200 - 10,000 dilutions)



WB using 1:1000 A010-50AP against 50 µg of rat brain homogenate +/- 1 min incubation with Ca<sup>2+</sup>/CaM/ATP.

PVDF membranes incubated with A010-50AP antibody : lot 0611-04 (2 µl in 2 ml TBS + 5% milk) 16 hours at 4°C.  
Secondary antibody = Goat anti Rabbit HRP. Chemiluminescent detection: 300 second exposure

**Related Products:** CaMKII Phospho Thr-286 epitope peptide (P010-50).

### Background References:

- Dobrev, D., & Wehrens, X.H.T. (2010) Trends in Cardiov. 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 Ca<sup>2+</sup>/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 b1-adrenergic stimulation modulates cardiac contractility by Ca<sup>2+</sup>/calmodulin kinase signaling pathway. Circ. Res. 95, 798-806.