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Phospholamban (PLN, PLB) (pSer16) pAb serum

Quality Control Certificate of Analysis
Catalogue No.:A010-12TV
Unit Size: 10 µL
Lot No: C6R1FB_Lyo260122

Background: Phospholamban (PLB/PLN) is a small transmembrane protein which plays an important role in controlling the activity of the sarcoplasmic reticulum ATPase (SERCA2a) of cardiac muscle during calcium sequestration (Drago and Colyer, 1994). Phospholamban is phosphorylated on separate amino acid residues by cAMP-dependent, and cGMP-dependent (Ser-16, Simmerman *et al*., 1986) and Ca^{2+}/CaM-dependent (Thr-17, Simmerman *et al*., 1986) protein kinases in response to β-adrenergic stimulation (Wegener *et al*., 1989). Akt has also been shown to phosphorylate Thr-17. The result is an increased calcium pump activity which reduces the time course of the calcium transient, increases the calcium load in the sarcoplasmic reticulum, and consequently, produces a larger calcium transient at the next action potential (Sham *et al*., 1991). However, alteration in this homeostatic interaction has been shown to result in heart failure (MacLennan and Kranias, 2003).

Description: Lyophilised **Rabbit** polyclonal anti-serum (A010-12TV) containing IgG antibody specific for Ser-16 phosphorylated forms of PLB (Drago & Colyer, 1994).

Immunogen: Phosphopeptide comprising residues 9-19-C (residues R_9SAIRRAS(PO_3H_2)TIE_{19}C) conjugated to KLH.

Antibody Isotype: IgG in antiserum.

Antibody Purity: Whole Serum.

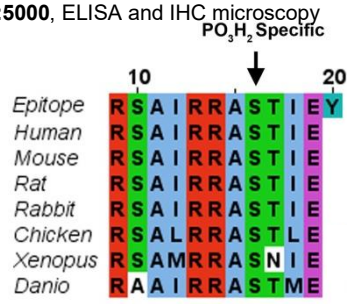
Specificity: The antibody recognises mono and oligomeric phospholamban when phosphorylated on serine-16 by PKA. Antiserum does not recognise unphosphorylated or Thr17 phosphorylated PLN; ~500-fold preference for pSer16-PLN. Antiserum does not recognise non-PLN proteins of molecular weight <40kDa in mouse ventricular myocytes (western blot data).

Species Cross Reactivity: Reacts with Phospho Ser-16 of PLB from Human, mouse, rat, rabbit, chicken, ferret, hamster and sheep.

Vial Constituents: Lyophilised A010-12TV Rabbit anti-serum (10 µl)

Storage Instructions: Lyophilised antibody is stable at 4 °C when stored with desiccant. Reconstitute lyophilised powder in 10 µl of 18 MΩ H_2O, aliquot and store frozen at -80 °C for 1 year. Avoid freeze - thaw cycles.

Tested Applications: **WB 1:5000**, ELISA and IHC microscopy



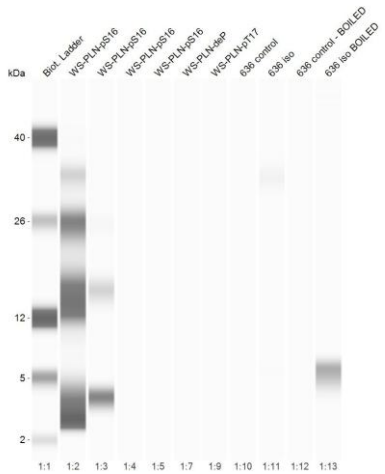


Figure 1: Serial dilution of pSer16-PLN (10ug/mL, 1ug/mL, 0.1ug/mL, 0.01ug/mL) recognised by A010-12 (1:50 dilution) in automated western blot. PLN and pThr17-PLN (10ug/mL) not recognised. Isoprenaline treated cardiac ventricular myocytes display pSer16-PLN in unboiled and boiled samples. Control myocytes display no pSer16-PLN

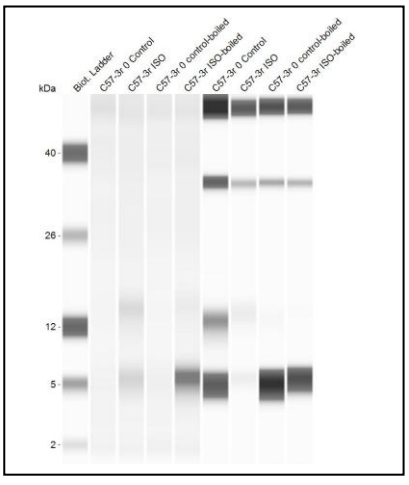


Figure 2: Mouse cardiac ventricular myocytes control and isoprenaline treated (400ug/mL) with and without heating to 95C (Boiled). PLN-pSer16 recognised by A010-12 (1:100 dilution) in automated western blot (first 4 lanes) and total PLN detected with A1 (A010-14, 1:35).

Background References:

- Drago, G. A., and Colyer, J. (1994) J Biol Chem 269, 25073-25077
- MacLennan, D. H., and Kranias, E. G. (2003) Nat Rev Mol Cell Biol 4, 566-577
- Sham, J. S., Jones, L. R., and Morad, M. (1991) Am J Physiol 261, H1344-1349
- Simmerman, H. K., Collins, J. H., Theibert, J. L., Wegener, A. D., and Jones, L. R. (1986) J Biol Chem 261, 13333-13341
- Wegener, A. D., Simmerman, H. K., Lindemann, J. P., and Jones, L. R. (1989) J Biol Chem 264, 11468-11474