

## Phospholamban (PLN, PLB) (pSer16) pAb

### Quality Control Certificate of Analysis

Catalogue No.: A010-12AP

Unit Size: 500 µg: in 100µL at 5mg/mL when dissolved

Lot No.: C6R1FBIgG-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<sup>2+</sup>/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 affinity purified Rabbit polyclonal antibody (A010-12AP) specific for Ser-16 phosphorylated forms of PLN (Drago & Colyer, 1994).

**Immunogen:** Phosphopeptide comprising residues 9-19-C (residues R<sub>9</sub>SAIRRAS(PO<sub>3</sub>H<sub>2</sub>)TIE<sub>19</sub>C) conjugated to KLH.

**Antibody Isotype:** IgG.

**Antibody Purity:** Protein A Affinity Purified. Total IgG from A010-12 serum (COL-6 rabbit1 FB).

**Specificity:** The antibody recognises mono and oligomeric phospholamban when phosphorylated on serine-16 by PKA. Antibody failed to detect dephos PLN or Thr17 phosphorylated PLN at 10µg/mL.

**Species Cross Reactivity:** Reacts with Phospho Ser-16 of phospholamban from mouse. Epitope is 100% identical in human, mouse, rat, rabbit, chicken.

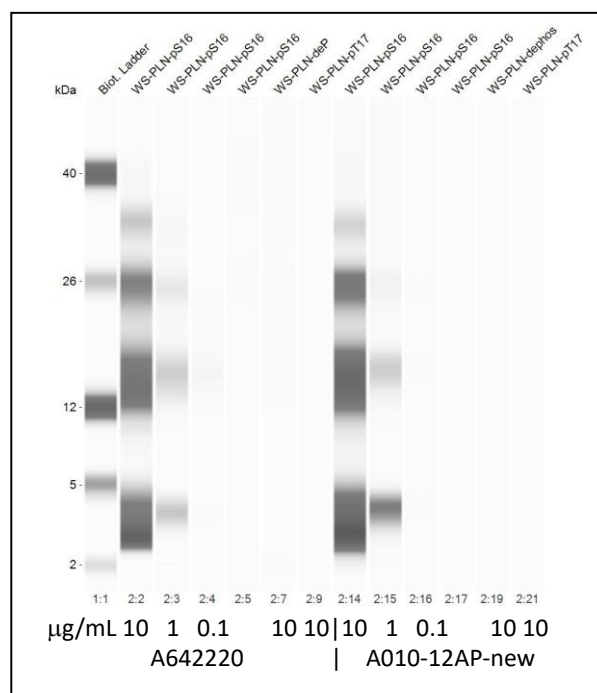
**Vial Constituents:** Lyophilised affinity purified A010-12AP Ab (100 µl) in PBS pH7.4, 0.02% sodium azide with 20%v/v stabiliser solution.

**Storage Instructions:** Lyophilised antibody is stable at 4°C when stored with desiccant. Reconstitute lyophilised powder in 100 µl of 18 MΩ H<sub>2</sub>O, aliquot and store frozen at -80°C for 1 year. Avoid freeze - thaw cycles.

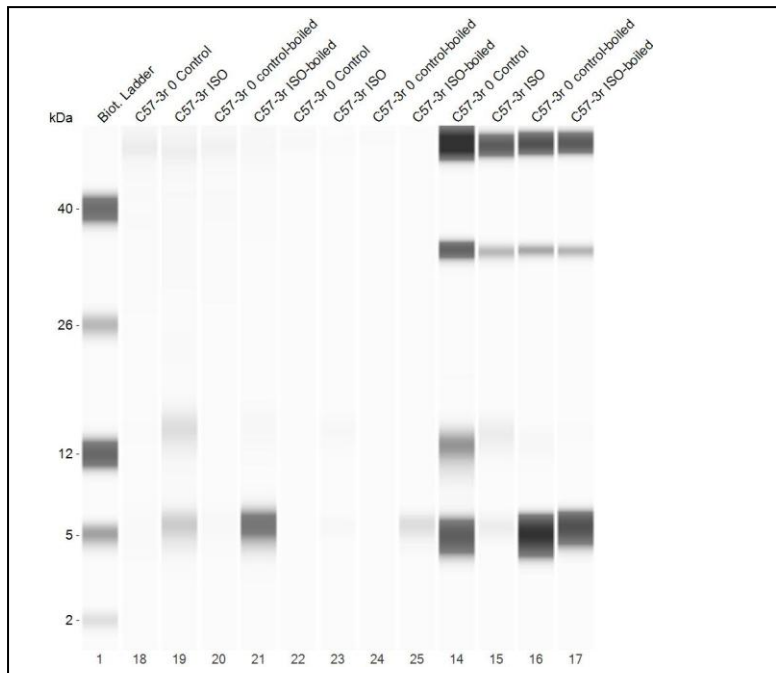
**Tested Applications:** WB JESS 1:10-1:25, WB manual ~1:1000

PO<sub>3</sub>H<sub>2</sub> Specific

|         | 10      | ↓     | 20 |
|---------|---------|-------|----|
| Epitope | RSAIRRA | ASTIE | Y  |
| Human   | RSAIRRA | ASTIE |    |
| Mouse   | RSAIRRA | ASTIE |    |
| Rat     | RSAIRRA | ASTIE |    |
| Rabbit  | RSAIRRA | ASTIE |    |
| Chicken | RSALRRA | ASTLE |    |
| Xenopus | RSAMRRA | ASNIE |    |
| Danio   | RAAIRRA | ASTME |    |



**Figure 1: Validation of specificity of A010-12AP for PLN-pSer16.** PLN-pSer16 was detected exclusively by the new and the previous preparation of A010-12AP. Dephos PLN and Thr17 phos PLN were not detected. A 10-fold serial dilution of full length PLN-pSer16 10µg/mL – 10ng/mL and 10µg/mL dephos-PLN and 10µg/mL PLN-pThr17 were analysed by automated western blotting JESS, at primary antibody concentration of 0.5mg/mL. Only PLN-pSer16 was detected. Specificity >500:1.



**Figure 2: Validation of specificity of A010-12AP for PLN-pSer16.** Mouse heart ventricular myocyte lysates; control and isoprenaline-treated were analysed by automated western blotting (JESS, ECL detection) with and without sample boiling. PLN (total) was detected using monoclonal antibody A1 (A010-14) in all 4 samples (lanes 14-17) as pentamer, dimer and monomer. PLN-pSer16 alone was detected by A010-12AP (C6R1FBIgG-Lyo260122; 1:10 dilution; lanes 18-21) in isoprenaline-treated samples, unboiled (dimer & monomer) and boiled (monomer). A previous lot of A010-12AP detected PLN-pSer16 (A642220; 1:10 dilution; lanes 22-25) in isoprenaline-treated samples, unboiled (monomer (weak)) and boiled (monomer). No non-specific binding to “off-target” proteins was seen in the molecular weight range analysed (2-40kDa) with A010-12AP antibody.

**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