

Phospholamban (PLN, PLB) mAb (clone A1)

Quality Control Certificate of Analysis

Catalogue No.: A010-14

Unit Size: 25 µg

Lot No.: A1-141017-251115

Background: The majority of calcium required for muscle contraction is mobilised from the Sarcoplasmic Reticulum (SR), to which it has to return to in order to facilitate relaxation. The (Ca^{2+} - Mg^{2+}) ATPase enzyme is responsible for the sequestration of calcium after its release from the SR, and is controlled by another sarcoplasmic reticulum protein called phospholamban (PLB) in cardiac, smooth and slow-twitch skeletal muscle (Drago and Colyer, 1994). The monoclonal antibody (A1) was raised against bovine cardiac PLB (Suzuki and Wang, 1986) and recognises an epitope comprising of residues γ LTRSAIRRAS₁₆ (Morris *et al.*, 1991). The antibody reacts with all forms of PLB: monomer, oligomers, phosphorylated and non-phosphorylated. The antibody stimulates Ca^{2+} pump activity upon binding to PLB, to an extent equivalent to stoichiometric phosphorylation of PLB (Jackson and Colyer, 1996). Manipulation of SERCA2:PLB ratios is a therapeutic strategy for heart failure. Elimination of PLB boosts SR Ca^{2+} -load which can exacerbate pathological outcomes (apoptosis, arrhythmias) when combined with other alterations in SR function.

Description: Affinity Purified Mouse monoclonal antibody (A010-14) to phospholamban.

Immunogen: Bovine cardiac phospholamban. Epitope has been mapped to residues 7-16 (Morris *et al* 1991).

Antibody Isotype: IgG.

Antibody Purity: Protein A affinity purified

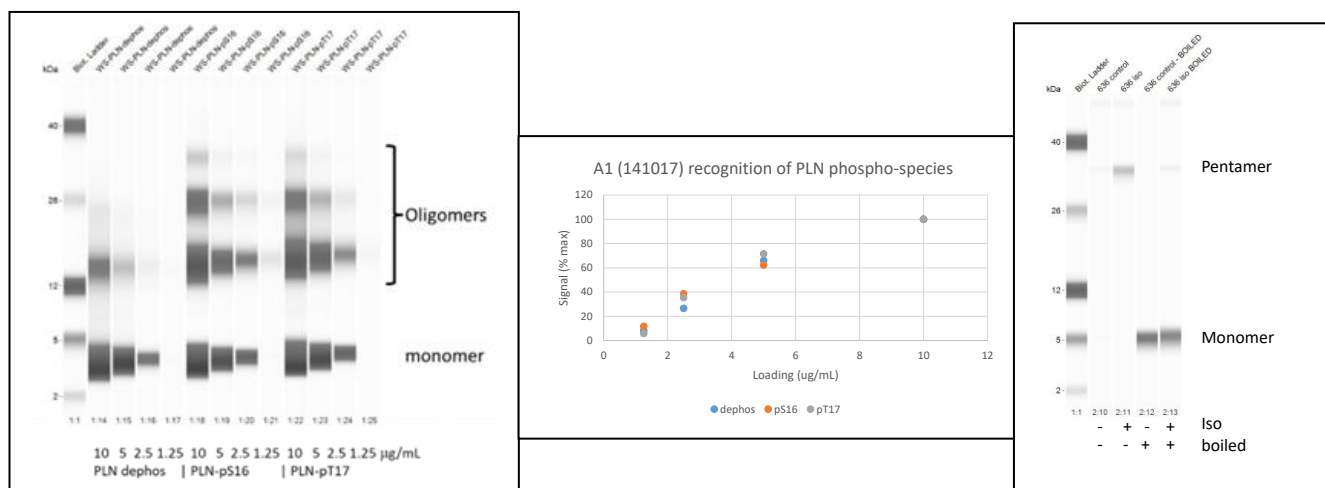
Specificity: The antibody recognises all forms of phospholamban: monomer, oligomer, phosphorylated and non-phosphorylated. Binding of the antibody to its target epitope is blocked in the presence of a peptide containing the PLB A1 epitope. The antibody is able to detect all phosphorylated forms of PLN similarly (Figures below)

Species Cross Reactivity: Reacts with phospholamban from bovine, canine, ferret, hamster, human, rat, mouse and sheep species

Vial Constituents: Freeze dried antibody (25µg) from a solution of PBS, pH 7.2 with sugar/polymer stabiliser at 20% by volume. Total volume 45 µL

Storage Instructions: Freeze dried antibody is stable at 4°C when stored with desiccant. Reconstitute pellet in 45µL of 18 MΩ H₂O. Aliquot and store frozen at -80°C for 1 year. Avoid freeze – thaw cycles.

Tested Applications: WB; recommended dilution 1:4000-1:10000; optimise accordingly.



Validation of A010-14 specificity: Automated western blots were performed with pure PLN protein in dephosphorylated, pS16 phosphorylated and pT17 phosphorylated forms (10µg/mL to 1.25µg/mL: left panel). The WB data were quantified and plotted as relative signal intensity (%max) showing equivalent sensitivity to all forms of PLN (middle panel). PLN was detected in mouse cardiac myocytes (0.4mg/mL) with and without isoprenaline treatment, with and without sample boiling (right panel). All data used A010-14 (lot A1-141017-251115) in ECL blotting at saturating concentration (20µg/mL). Manual WB uses much higher dilutions of primary antibody (~1:5000).

Background References:

- Drago, G. A., and Colyer, J. (1994): *J Biol Chem* **269**, 25073-7.
- Jackson & Colyer (1996): *Biochem. J.* **316**, 201-207.
- Morris *et al.* (1991): *J. Biol. Chem.* **266**, 11270-11275.
- Suzuki & Wang (1986): *J. Biol. Chem.* **261**, 7018-7023.