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### A010-31 anti-RYR2 phospho-Ser2814 50µl

**Background:** The ryanodine receptor (RyR2) is a  $\text{Ca}^{2+}$  channel of cardiac muscle that plays a central role in EC coupling. The binding of  $\text{Ca}^{2+}$  to RyR2 opens the channel and  $\text{Ca}^{2+}$  stored in the SR moves through the channel into the cytosol to initiate muscle contraction (Bers, 2002). CaMKII, was able to phosphorylate Ser-2814 of RYR2 (Wehrens et al., 2004) enhancing  $\text{Ca}^{2+}$ -sensitivity and increasing open probability.

**Description:** Lyophilised rabbit polyclonal serum containing IgG antibody specific to RyR2 phospho-Ser2814 (CaMKII site).

**Immunogen:** Synthetic peptide (TSQVS(PO<sub>3</sub>H<sub>2</sub>)VDAAH<sub>2819</sub>) corresponding to amino acids surrounding the phosphorylated serine residue at position 2814 of RYR2 (human). Sequence corresponds exactly to Ser-2815 in rabbit. Sequence in mouse & rat differ at one residue (V2815I), however antibody recognises both sequences.

**Specificity and Species Cross Reactivity:** The antibody recognises phosphorylated serine 2814 of the ryanodine receptor and binding is blocked in the presence of a peptide containing the phospho-Ser2814 epitope. The antibody reacts with phos-Ser2814 of ryanodine receptor from human, rat, mouse, rabbit, canine, and sheep species despite the single residue difference between some of these species (V2815I).

**Applications:** Western blot (1:5000 dilution), Immunofluorescence microscopy (1:100)

**Storage:** Store antibody desiccated at 4C when dry, and frozen (-20C or -80C) in small aliquots when reconstituted with 50µl deionised water.

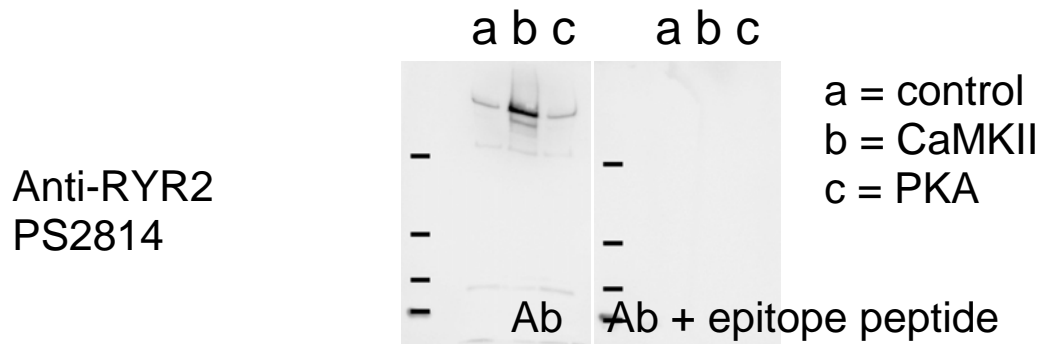
#### Epitope sequence alignment RYR2 (2810-2819)

|                 |                          |
|-----------------|--------------------------|
| Epitope         | TSQVSVDAAH               |
| Human Ser-2814  | TSQVSVDAAH               |
| Mouse Ser-2814  | TSQVSI <sup>I</sup> DAAH |
| Rabbit Ser-2815 | TSQVSVDAAH               |
| Rat Ser-2814    | TSQVSI <sup>I</sup> DAAH |

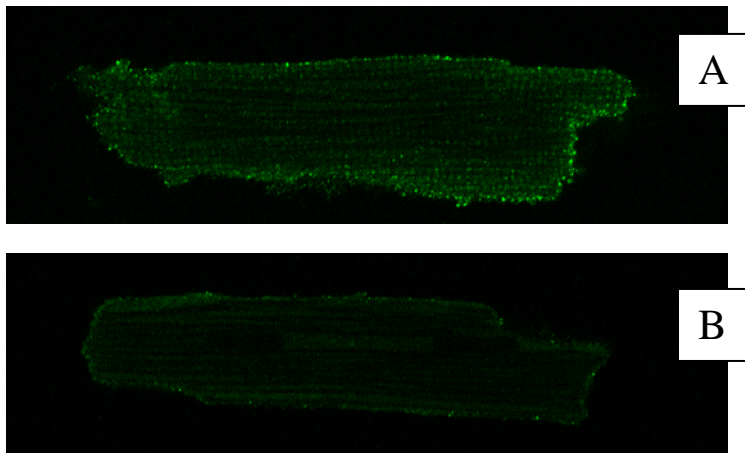
#### references:

Bers, D. M. (2002) *Nature* 415, 198-205.

Wehrens, X.H.T., Lehnart, S.E., Reiken, S.R. & Marks, A.R. (2004) *Circ. Res.* 94, e61-e70



**Figure 1: Detection of RYR2 phosphorylated at Ser2814 in rat cardiac SR** Cardiac SR vesicles were phosphorylated by CaMKII (sample b) or PKA (sample c) for 1 min at 37C. Control vesicles were incubated without kinase or ATP (a). 10µg protein was separated by 6% SDS-PAGE electrophoresis and transferred to PVDF membrane. Ser-2814 phosphorylated RYR2 was detected with anti-RYR2 phospho-Ser2814 (A010-31; 1:1000) detected with peroxidase based chemiluminescence. Similar results were obtained with 1:2000 dilution of A010-31. Epitope peptide (P010-31, 1µM) abolished all immunorecognition, indicating that all staining is specific for phospho-2814 RYR2. The position of molecular weight markers at 175kDa, 83kDa, 66kDa and 45kDa are marked on the figure.



**Figure 2 Detection of Ser-2814 phosphorylated RYR2 by immunofluorescence microscopy.** Rat cardiac myocytes were stimulated electrically (0.5Hz) and with  $\beta$ 1-adrenergic agonist (100nM isoproterenol + 100nM ICI118551) for 5 minutes. Cells were fixed in 4% formaldehyde for 30min, washed in PBS (3 times) and permeabilised in 0.1% Triton X-100 in PBS. Non-specific binding sites were blocked with donkey serum, and cells were incubated with anti-RYR2 phospho-Ser2814 antibody (A010-31: panel A) at 1:100 dilution in the absence or presence of 3µM epitope peptide (P010-31: panel B) for 60min at room temperature. Cells were washed 3 times in PBS and incubated with fluorescently labelled secondary antibody (Alexa Fluor donkey anti-rabbit IgG, 1:500) for 2 hours. Cells were washed (3xPBS) and mounted on a slide and viewed under a confocal fluorescence microscope under oil immersion.