



**Badrilla**, Leeds Innovation Centre, 103  
Clarendon Road, Leeds LS2 9DF, UK

Tel: +44-(0)1937-572179  
Fax: +44-(0)1937-573168  
e-mail: info@badrilla.com

## A010-30 anti-RYR2 phospho-Ser2808 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). Abnormal structure and function of ryanodine receptors has been reported in failing hearts, with Ser-2808 phosphorylation appearing elevated in clinical situations which may contribute to the abnormal  $\text{Ca}^{2+}$  handling characteristics of cardiac muscle in these conditions (Wehrens and Marks, 2003). Ser-2808 can be phosphorylated in vitro by PKA or CaMKII (Rodriguez et al., 2003), which is coincident with significant change in RyR2 channel function typified by an increased open probability (Carter et al., 2006; Witcher et al., 1991; Valdivia et al., 1995; Marx et al., 2000), the abrogation of the inhibitory effects of CaM (Witcher et al., 1991) and  $\text{Mg}^{2+}$  (Hain et al., 1995), dissociation of regulatory factors, expression of subconductance states and the expression of channel activity at diastolic  $\text{Ca}^{2+}$  concentrations (Marx et al., 2000).

**Description:** Lyophilised rabbit polyclonal serum containing IgG antibody specific to RyR2 phospho-Ser2808. In a survey of available antibodies, Badrilla's anti-RYR2 phospho-Ser2808 was described as "most reliable to detect phosphorylation" by Huke & Bers (2008), the only antibody which "always detected" changes in phosphorylation status of RYR2.

**Immunogen:** Synthetic peptide (YNRTRRIS(PO<sub>3</sub>H<sub>2</sub>)QT<sub>2810</sub>) corresponding to amino acids surrounding the phosphorylated serine residue at position 2808 of RYR2 (human). **Sequence corresponds exactly to Ser-2809 in rabbit.**

**Specificity and Species Cross Reactivity:** The antibody recognises phosphorylated serine 2808 of the ryanodine receptor and binding is blocked in the presence of a peptide containing the phospho-Ser2808 epitope. The antibody reacts with phos-Ser2808 of ryanodine receptor from human, rat, mouse, rabbit, canine, rat and sheep species.

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

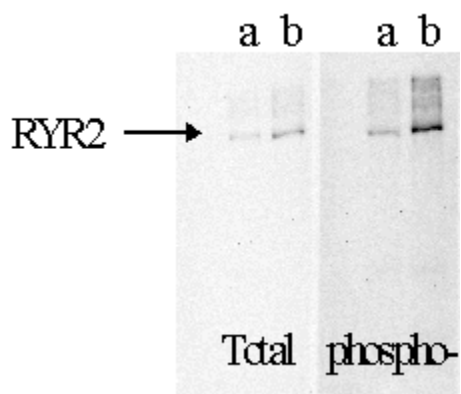
**Storage:** Store antibody desiccated at 4C when dry, and frozen (-20C or -80C) in small aliquots when reconstituted with 50µl deionised water.

### Epitope sequence alignment RYR2 (2801-2810)

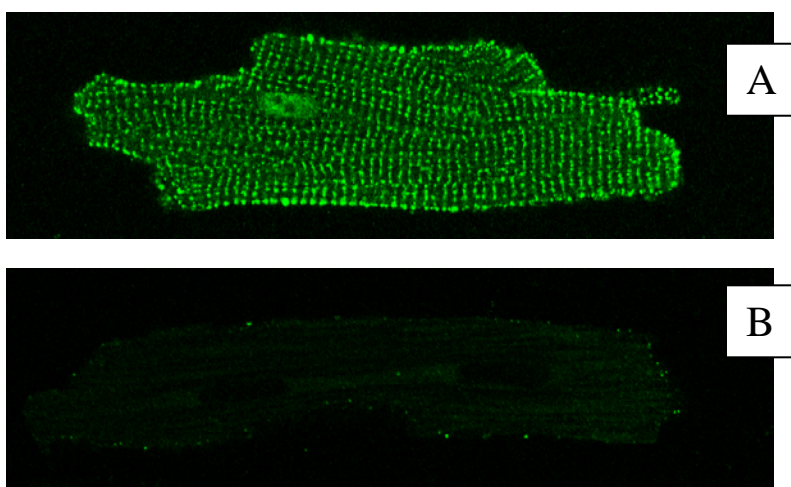
Epitope	YNRTRRISQT
Human Ser-2808	YNRTRRISQT
Mouse Ser-2808	YNRTRRISQT
Rabbit Ser-2809	YNRTRRISQT
Rat Ser-2808	YNRTRRISQT

### references:

Bers, D. M. (2002) *Nature* 415, 198-205.  
Carter, S., Colyer, J., & Sitsapesan, R. (2006) *Circ. Res.* 98, 1506-13  
Hain, J., Onoue, H., Mayrleitner, M., Fleischer, S., and Schindler, H. (1995) *J Biol Chem* 270, 2074-81.  
Marx, S. O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosembliit, N., and Marks, A. R. (2000) *Cell* 101, 365-76.  
Rodriguez, P., Bhogal, M. S., and Colyer, J. (2003) *J Biol Chem* 278, 38593-600.  
Valdivia, H. H., Kaplan, J. H., Ellis-Davies, G. C., and Lederer, W. J. (1995) *Science* 267, 1997-2000.  
Wehrens, X. H., and Marks, A. R. (2003) *Trends Biochem Sci* 28, 671-8.  
Witcher, D. R., Kovacs, R. J., Schulman, H., Cefali, D. C., and Jones, L. R. (1991) *J Biol Chem* 266, 11144-52.



**Figure 1: Detection of RYR2 phosphorylated at Ser2808 in rat cardiac SR** (Cardiac SR vesicles were phosphorylated by CaMKII for 1 min at 37C (sample b). Control vesicles were incubated without kinase or ATP (a). 10µg protein was separated by 6% SDS-PAGE electrophoresis and transferred to PVDF membrane. Total RYR2 was detected with antibody 34C (Total), Ser-2808 phosphoylated RYR2 was detected with anti-RYR2 phospho-Ser2808 (A010-30: 1:5000) detected with peroxidise based chemiluminescence.



**Figure 2 Detection of Ser-2808 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-Ser2808 antibody (A010-30: panel A) at 1:100 dilution in the absence or presence of 3µM epitope peptide (P010-30: 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.