

Ryanodine Receptor 2 (RyR2) (pSer2814) pAb

Quality Control Certificate of Analysis

Catalogue No.: A010-31

Unit Size: 50 µl

Lot No: 1209-04

Background: The ryanodine receptor (RyR2) is a Ca²⁺ channel of cardiac muscle that plays a central role in EC coupling. The binding of Ca²⁺ to RyR2 opens the channel and Ca²⁺ 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 Ca²⁺- sensitivity and increasing open probability. Excessive phosphorylation of Ser-2814 leads to atrial fibrillation (Dobrer and wehsens, 2010), arrhythmias and sudden death (Van Oort et al, 2010)

Description: Lyophilised **Rabbit** anti-serum (A010-31)

Vial Constituents: Lyophilised A010-31 Rabbit anti-serum (50 µl)

Immunogen: Synthetic peptide (TSQVS(PO₃H₂)VDAAH₂₈₁₉) corresponding to amino acids surrounding the phosphorylated serine residue at position 2814 of RyR2 (human) conjugated to KLH.

Storage Instructions: Lyophilised antibody is stable at 4°C when stored with desiccant. Reconstitute lyophilised powder in 50 µl of 18 MΩ H₂O, aliquot and store frozen at -80°C for 1 year. Avoid freeze - thaw cycles.

Antibody Isotype: IgG.

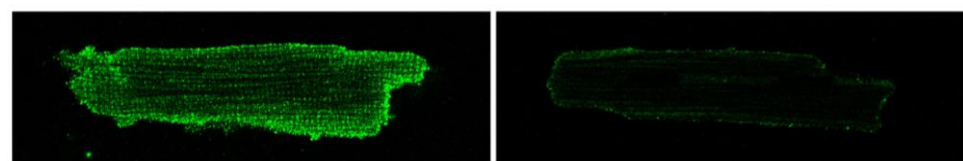
Tested Applications: WB 1:5000, IHC 1:100

Antibody Purity: Raw Serum.

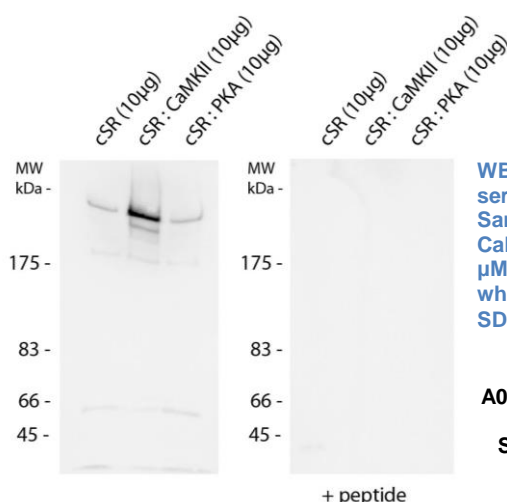
Specificity: Recognises Ser-2814 phosphorylated RyR2 exclusively, and will not react with dephosphorylated RyR2 or RyR2 phosphorylated at other sites.

Species Cross Reactivity: Peptide aligns with RyR2 from all mammalian species. Sequence corresponds exactly in rabbit but differs at one residue (V2815I) in mouse and rat. However antibody recognises both sequences.

	PO ₃ H ₂ Specific											
	2810 ↓ 2820											
Epitope	-	T	S	Q	V	S	V	D	A	A	H	-
Human	Q	T	S	Q	V	S	V	D	A	A	H	G
Mouse	Q	T	S	Q	V	S	I	D	A	A	H	G
Rat	Q	T	S	Q	V	S	I	D	A	A	H	G
Rabbit	Q	T	S	Q	V	S	V	D	A	A	H	G
Dog	Q	T	S	Q	V	S	V	D	A	A	H	G
Danio	L	S	S	Q	R	S	I	E	G	A	H	G



IHC Microscopy using 1:100 RYR2 Phospho Ser-2814 anti-serum (A010-31) against Rat cardiac myocytes stimulated electrically at 0.5Hz and chemically with β1-adrenergic agonist (100nM isoproterenol + 100nM ICI118551) for 5 minutes. Cells were fixed in 4% formaldehyde for 30min, washed in PBS (3 times) and permeabilised using 0.1% Triton X-100 in PBS. Cells were blocked with donkey serum, and incubated with 1:100 anti-RYR2 Phospho Ser-2814 antibody (A010-31) +/- 3µM epitope peptide for 60min at room temperature. Cells were washed 3 times in PBS and incubated with 1:500 Alexa Fluor donkey anti-rabbit IgG for 2 hours. Cells were washed (3xPBS) and mounted on a slide and viewed under a confocal fluorescence microscope under oil immersion.



WB using 1:5000 RYR2 Phospho Ser-2814 anti-serum (A010-31) against 10µg of canine cardiac Sarcoplasmic Reticulum +/- Protein Kinase A or CaMKII treatment. Blots run in duplicate +/- 1 µM immunising peptide. A010-31 detects RYR2 when phosphorylated by CaMKII (350 kDa) 6 % SDS-PAGE gel. PVDF membrane

**A010-31 (1:5000) 1 µl in 5 ml of Tris buffer + 5% milk powder 16 hours at 4°C.
Secondary antibody = Goat anti Rabbit HRP
Chemiluminescent detection**

Related Products: A010-30AP RYR2 Phospho Ser-2808 (AP), A010-32AP RYR2 Phospho Ser-2030 (AP); A010-35AP RYR2 Dephospho Ser-2808 (AP), A010-31AP RYR2 Phospho Ser-2814 (AP).

Background 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
- Dobrer, D. & Wehrens, X.H.T (2010) *Trends in Cardiovascular Medicine.* 20, 30-34
- Van Oort, R.J et al. (2010) *circulation.* 122, 2669-79