

ABCD_RB932 to ABCD_RB937 antibodies recognize amino acids 56 to 120 of the human cardiac troponin T isoform 6 by ELISA

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Abstract

ABCD_RB932, ABCD_RB933, ABCD-RB934, ABCD_RB935, ABCD_RB936 and ABCD_RB937 antibodies were generated against a peptide corresponding to amino acids 56-120 of the human cardiac troponin T isoform 6. All the discovered antibodies were able to recognize the peptide by ELISA. Antibodies ABCD_RB932, ABCD_RB934, ABCD_RB935 and ABCD_RB937 are also able to bind the full-length cardiac troponin T and the cardiac troponin ITC complex.

Introduction

Cardiac troponin T (cTnT, UniProt #P45379) is a cytosolic protein essential for the contraction and relaxation of cardiomyocytes. In normal adult human heart tissue, the predominant splice isoform of cTnT is isoform 6, also referred to as cTnT3. cTnT functions as part of the troponin complex (ITC), which also includes cardiac troponin I (cTnI) and troponin C (cTnC). The detection of circulating cTnI and cTnT in blood samples via ELISA is considered the gold standard for diagnosing myocardial cell injury (Thygesen *et al.*, 2019). In blood, cTnT can be present as different circulating proteoforms (e.g., full-length protein, proteolytic fragments, and complexed forms). Selective identification of different circulating cTnT proteoforms may help to differentiate between myocardial infarction and non-ischemic causes of myocardial injury (Airaksinen *et al.*, 2022). The aim of this study was to evaluate new recombinant antibodies, selected against a peptide corresponding to amino acids 56–120 of cTnT splice isoform 6, for their ability to recognize different circulating proteoforms of cTnT by ELISA.

Materials & Methods

Antibodies: The recombinant antibodies ABCD_RB932, ABCD_RB933, ABCD-RB934, ABCD_RB935, ABCD_RB936 and ABCD_RB937 (ABCD

nomenclature, <http://web.expasy.org/abcd/>, referred to collectively as RB932-937) were generated by the Geneva Antibody Facility (<http://unige.ch/medecine/antibodies/>). Briefly, a synthetic VHH phage display library (in-house) was panned against the C-terminally biotinylated peptide corresponding to amino acids 56-120 of the human cTnT isoform 6 (sequence: EDGPM EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRME KDLNELQALIEAHFENRKKEEEEEL, PeP89). After three rounds of panning, selected phage vectors were isolated using a plasmid preparation kit (Qiagen), and the VHH inserts were subcloned into custom-made expression vectors and sequenced. The selected antibodies were expressed as VHH domains fused to a human IgG1 Fc region. HEK293 suspension cells, cultured in HEK TF chemically defined, animal-component-free and serum free medium (#861-0001, Sartorius) supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vectors encoding the VHH-Fc constructs. Supernatants containing antibodies were harvested three days post-transfection. Production yields and purity were estimated by Coomassie staining. All antibodies displayed a clearly defined band of the expected size, and no protein contamination was observed (data not shown). Production yield was approximately 20 mg/L for antibodies RB936, between 35–45 mg/L for antibodies RB932 and RB933 and between 85–110 mg/L for RB934, RB935, RB937 and RB938.

Antigen: The antibodies were tested by ELISA against the targets described in table 1. The antibodies were tested by ELISA against the targets described in table 1. All the peptides were synthesized and C-terminally biotinylated the UNIGE Peptide Synthesis Core Facility. The recombinant human full-length cTnT protein (cTnT, purity >95%) and ITC complex (purity >95%) were purchased from HyTest (#8RTT5 and #8ITCR respectively). The human cTnI protein was purchased from Sigma (#T9924). The human skeletal TnT (skTnT) was purchased by Hytest (#8T24).

ELISA: The whole procedure was carried out at room temperature. Biotinylated peptides, full length proteins and ITC complex at saturating concentration (10 pmol/well) were immobilized on maxiSorp ELISA plates (Nunc #442404) for 30 min. Each well was rinsed three times with 100 μ l of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated overnight with 50 μ l of RB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 μ l washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-human IgG (Sigma #A8275, dilution 1:1000, 50 μ l per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 μ l per well). The reaction was stopped by the addition of 25 μ l of 2 M H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Table 1: Description of the antigens used in the ELISA assay.

Name	Description/sequences
Control	Peptide from zebrafish fibrinogen alpha protein (UniProt # Q6DHS2) KFSTFDRDNDKWEENC
PeP51	Peptide from human cTnT (UniProt #P45379-6) Residues 1-66 MSDIEEVVEEYEEEEQEEAAVEEQEEAAEE DAEAEAETEETRAEEDEEEEEAKEAEDGPM EESKPK
PeP89	Peptide from human cTnT (UniProt #P45379-6) Residues 56-120 EDGPMEEKPKPRSFMPNLVPPKIPDGERVD FDDIHRKRMEKDLNELQALIEAHFENRKKKE EEL
PeP90	Peptide from human cTnT (UniProt #P45379-6) Residues 111-175 ENRKKEEELVSLKDRIERRRAERAEQQRIR NEREKERQNRLAEERARREEEENRRKAEDE ARKK
PeP91	Peptide from human cTnT (UniProt #P45379-6) Residues 230-298 AIDHLNEDQLREKAKELWQSIYNLEAEKFD LQEKFKQKYEINVLNRNINDNQKVKSTRG KAKVTGRWK
skTnT	Human skeletal TnT (UniProt #P45378) Full length
cTnI	Human cardiac TnI (UniProt #P19429) Full length
cTnT	Human cTnT (UniProt #P45379-6) Full length
ITC	cTnI, cTnT and cTnC complex

Results and discussion

Antibodies RB932 to RB937 were evaluated by ELISA against peptides derived from various regions of cTnT (PeP51, PeP89, PeP90, PeP91). As shown in Figure 1, all tested antibodies exhibited concentration-dependent binding to PeP89, the peptide against which they were originally selected. In contrast, no significant binding was

observed to the negative control peptide (PeP66) or to the other cTnT-derived peptides (Fig. 1). The relatively lower signal observed for RB936 may be due to its lower concentration in the assay. The RB933 binding curve suggests a lower affinity.

To further characterize the antibodies, their ability to recognize full-length cTnT and the cardiac troponin complex (ITC), which includes cTnT, cTnI and cTnC, was assessed. The results showed that RB932, RB934, RB935, and RB937 bound to both free cTnT and the ITC complex. Importantly, none of the tested antibodies exhibited binding to other troponin proteins, including cTnI and skeletal troponin T (skTnT), confirming their specificity for cTnT.

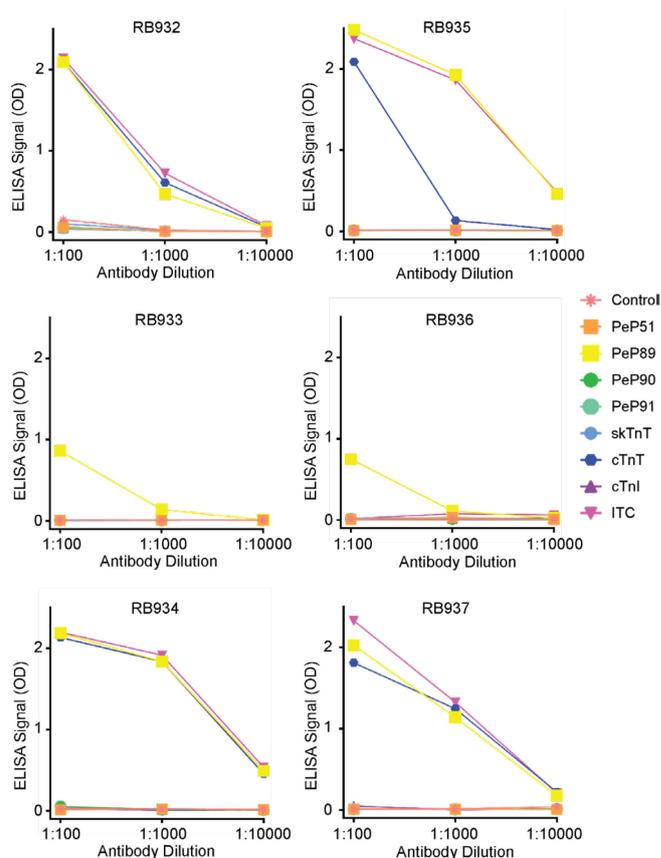


Fig. 1. Specific binding of RB antibodies to the target PeP89 peptide, as detected by ELISA. Refer to Table 1 for a description of the different antigens tested.

Our novel antibodies are specific for PeP89, which comprises an amino acid sequence distinct from the epitope recognized by the only commercially available cTnT assay (Roche Diagnostics). The Roche assay targets an amino acid sequence within the peptide 90 region, using a capture antibody directed against residues 125–131 and a detection antibody directed against residues 136–147. The clinical relevance and potential added value of separately detecting these peptides warrant further investigation across different pathological and clinical contexts. In comparison with commercially available antibodies, the newly developed recombinant antibodies offer several important advantages. Their generation

ensures sustained availability for future experiments and increases confidence in antibody specificity, as their molecular targets and binding characteristics are fully characterized. Moreover, once isolated, these recombinant antibodies can be produced at very low cost, making them economically advantageous in the long term. Importantly, their distribution to the scientific community promotes open science and enhances the reproducibility of research findings. This strategy may provide greater flexibility with respect to intellectual property, thereby facilitating the potential translation of our results into a commercial diagnostic assay or biomarker. Nevertheless, the analytical performance of a fully developed diagnostic test based on these recombinant antibodies, including sensitivity, dynamic range, and limit of detection, remains to be established. Future studies should therefore systematically evaluate these parameters and compare the performance of recombinant antibody-based assays with existing commercially available tests.

References

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Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.