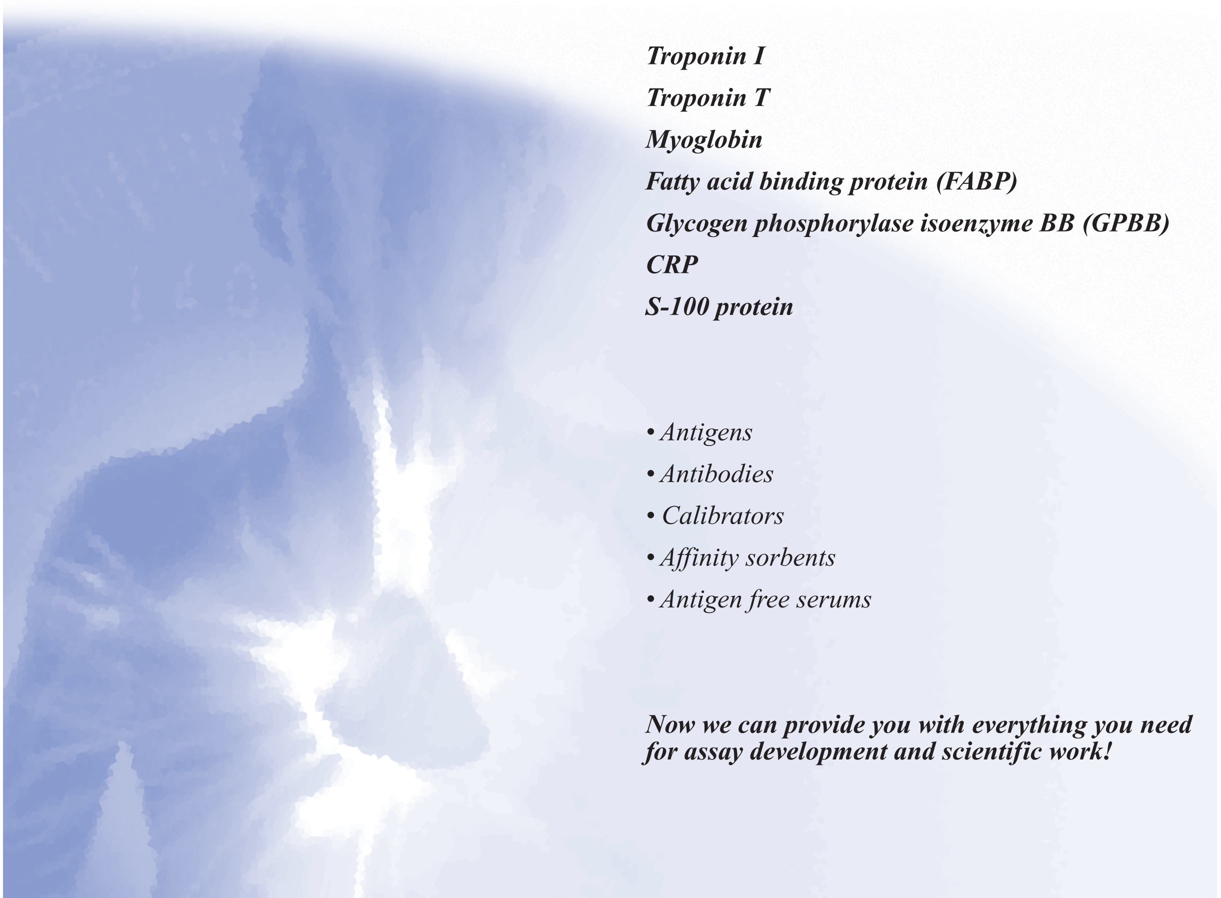
Cardiac Markers Panel

Introduction

Cardiovascular diseases (CVD) are the most lethal diseases in the Western world. When the standard of living is growing all the time CVD are going to be the leading cause of mortality in the whole world in the near future. This means more demanding challenges to scientific communities to research and develop new and more specific diagnostics approaches to unstable angina and acute myocardial infarction (AMI). In this field different cardiac markers have already today a major role but the demand for more effective and sensitive cardiac markers is widely recognized in the whole diagnostic world.

For more than 10 years HyTest specialists have been involved in the development and production of high quality cardiac markers. We have concentrated our research on the importance of different markers as a cardiac specific marker in order to help the scientific and industrial communities to conduct their own research and product development in the field of CVD diagnostics. Today we can proudly say that HyTest is one of the leading producers of different reagents for cardiac diagnostic. We are constantly widening the product range and encouraging contacs with our clients.

The purpose of this booklet is to present additional information about our products according to the following groups:



Troponin I

Troponin T

Myoglobin

Fatty acid binding protein (FABP)

Glycogen phosphorylase isoenzyme BB (GPBB)

CRP

S-100 protein

- *Antigens*
- *Antibodies*
- *Calibrators*
- *Affinity sorbents*
- *Antigen free serums*

Now we can provide you with everything you need for assay development and scientific work!

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I Proteins of troponin complex

Troponin complex – a heteromeric protein playing an important role in the regulation of skeletal and cardiac muscle contraction. Troponin complex consists of three different subunits – troponin T (TnT), troponin I (TnI) and troponin C (TnC). Each subunit is responsible for the part of troponin complex function. TnT is a tropomyosin-binding subunit, regulates the interaction of troponin complex with thin filaments; TnI inhibits ATP-ase activity of acto-myosin and TnC is a Ca^{2+} - binding subunit, playing the main role in Ca^{2+} dependent regulation of muscle contraction.

TnT and TnI in cardiac muscle are presented by forms different from those in skeletal muscles. Two isoforms of TnI and two isoforms of TnT are expressed in human skeletal muscle tissue (skTnI and skTnT). Only one tissue-specific isoform of cTnI is described for cardiac muscle tissue (cTnI), whereas latest publications indicated the existence of at least four cardiac specific isoforms of TnT (cTnT). But possibly much more cardiac specific isoforms of TnT can be expressed in myocard. There is no cardiac –specific isoform described for human TnC. TnC in human cardiac tissue is presented by an isoform typical for slow skeletal muscle. Another – fast skeletal TnC isoform is more typical for fast skeletal muscles.

cTnI is expressed only in myocardium. No examples of cTnI expression in healthy or injured skeletal muscle or in other tissue types are described. cTnT probably is less cardiac specific. Expression of cTnT in skeletal tissue of the patients with chronic skeletal muscle injuries was described.

First cTnI (in 1987) and later cTnT (in 1989) were used as markers of cardiac cell death. Now both proteins are widely used for the diagnosis of acute myocardial infarction (AMI), unstable angina, post-surgery myocardium trauma and some other diseases related with cardiac muscle injury, gradually replacing the “golden marker” of the last decade – CKMB. Both markers can be detected in patient’s blood 3 – 6 hours after onset of the chest pain, reaching peak level within 16 – 30 hours. Elevated concentration of cTnI and cTnT in blood samples can be detected even 5 – 8 days after onset of the symptoms, making both proteins useful also for the late diagnosis of AMI.

1. Troponin I

1.1. Human cardiac troponin I (cTnI)

Source:	*human cardiac tissue*
Purity:	*≥ 95 %*
Presentation:	*lyophilized*
Application:	*immunogen for antibody production, mass cTnI standard, cTnI biochemical and immunochemical studies*
Handling:	*reconstitute in deionized water*
Remarks:	*cTnI has poor solubility in buffers with physiological salt concentrations and pH values*

Human cardiac troponin I is presented in cardiac tissue by a single isoform with molecular weight 23876 Da and consists of 209 amino acid residues. The theoretical pI of cTnI is 9.87.

cTnI molecule contains two serines in the 22 and 23 positions. Both amino acid residues can be phosphorylated *in vivo* by protein kinase A, so four forms of protein - one dephospho two monophospho- and one bisphospho- can coexist in the cell. Phosphorylation of cTnI changes the conformation of the protein and modifies its interaction with other troponins as well as the interaction with anti-TnI antibodies. According to the latest findings significant part of cTnI released into the patient’s blood stream is phosphorylated.

For more than one decade cardiac form of TnI (cTnI) is known as a reliable marker of cardiac tissue injury. It is considered to be more sensitive and significantly more specific in diagnosis of the myocardial infarction than the “golden marker” of last decade – CK-MB, as well as myoglobin and LDH isoenzymes.

cTnI is purified from human cardiac tissue by immunoaffinity method followed by additional ion-exchange chromatography. Preparation contains some amount (<5 %) of cTnI proteolytical fragments retaining troponin’s immunological activity. According to immunological and mass spectral studies cTnI is acetylated (from N-terminal part of the molecule) and partially mono- and biphosphorylated. cTnI isolated from cardiac tissue and containing

posttranslational chemical modifications represents more “natural” form of the protein comparing with highly purified recombinant cTnIs.

On SDS-PAGE cTnI is presented by a single band with apparent molecular weight 29 kDa (Fig. 1, Lane 3).

Completely phosphorylated (biphosphorylated) and dephosphorylated forms of natural cTnI can be prepared on the customer request.

Ordering information

Product	Cat #	Purity	Source
Troponin I Cardiac, Human	**8T53**	>95 %	Human Cardiac Muscle
Phosphorylated cardiac Troponin I	**8T52ph**	>95 %	Human Cardiac Muscle
Dephosphorylated cardiac Troponin I	**8T52dp**	>95 %	Human Cardiac Muscle

1.2. Troponin complex

Source: *human cardiac tissue*
Composition: *cTnI, cTnT, TnC*
Presentation: *lyophilized or frozen solution*
Application: *stabilized form of natural cTnI, best for the calibration of the assays and standard preparation, immunogen for antibody production, troponin biochemical and immunochemical studies*

Inside the cardiac troponin complex the strongest between-molecule interaction was demonstrated for cTnI – TnC binary complex especially in the presence of Ca^{2+} ($K_A = 1.5x10^{-8}$ M^{-1}). This interaction is very important for cTnI immunodetection and should be considered by the assay manufacturers. TnC, forming complex with cTnI, changes the conformation of cTnI molecule and shields part of it’s surface, thus affecting the interaction of some anti-cTnI antibodies with the antigen. Hence the immunological properties of cTnI in troponin complex are considerably different from the properties of the free protein. As a result – some MAbs recognising free (isolated) cTnI do not interact with cTnI in cTnI-TnC binary or cTnI-cTnT-TnC ternary complexs. According to the latest data cTnI is released in the blood stream of the patient in the form of binary complex with TnC or ternary complex with cTnT and TnC.

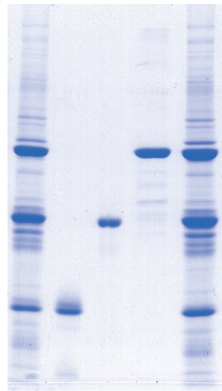
At the same time cTnI-TnC complex formation plays an important positive role, improving the stability of cTnI molecule. cTnI, which is an extremely unstable in a free form, demonstrates significantly better stability in complex with TnC or in ternary cTnI-cTnT-TnC complex. These two forms of the protein are preferable as a material for standard and calibrator preparation.

In 1996 HyTest released a new product - the native human cardiac troponin complex (Fig 1 - Lanes 1 and 5). In troponin complex cTnI is presented in the same form as it can be detected in the blood of the AMI patients. Purification of the troponin complex is performed in mild conditions without treatment of the proteins with urea containing buffers (as it is usually done for the preparation of the individual troponin components). The concentration is precisely determined for each of the three components of the complex (Table 1). It was demonstrated that stability of cTnI in native complex is significantly better than stability of the purified form of the protein or the stability of cTnI in artificial troponin complexes combined from purified proteins (Fig. 2).

On SDS-PAGE troponin complex is presented by a three main bands –cTnT, cTnI and TnC with corresponding apparent molecular weights 39, 29 and 18 kDa.

Table 1. Human cardiac troponin complex Lot 11/96 (example)

Troponin subunits	Concentration mg/ml	Molar Ratio
CTnI	0.49	1
TnC	0.53	1.29
TnT	0.53	0.7



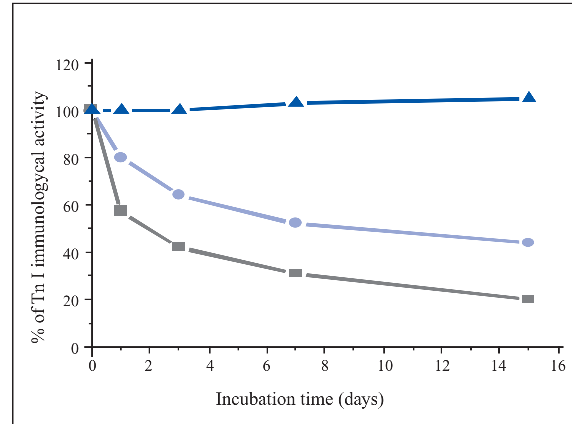


Fig. 1. SDS – gel electrophoresis of human cardiac troponin complex in reducing conditions. From left to right: lanes 1 and 5 - human cardiac troponin complex; lane 2 – human TnC; lane 3 – cTnI; lane 4 - cTnT.

Fig. 2. Comparison of the stability of the different forms of cTnI, dissolved in normal human serum and incubated for different periods of time at +4°C.
- ▲ - native troponin complex
- ● - artificial troponin complex combined from individual cTnI, cTnT and TnC
- ■ - purified cTnI

Advantages:

- antigen is in the same form as in AMI blood samples
    - unchanged tertiary structure
    - unchanged antibody binding sites
    - best to use for calibrators and standard preparation
- high cTnI stability
- determined concentration and molar ratio of all troponin components

Ordering information

Product	Cat. #	Purity	Source
Troponin Complex (I-T-C), Human	**8T62**	N/A	Human Cardiac Muscle

1.3. Calibrator set

Antigen: *cTnI in ternary troponin complex*
Antigen stability: *high*
Presentation: *lyophilized*

cTnI calibrators are based on isolated natural troponin complex and cover range of concentrations from 0 to 100 ng/ml. Each calibrators lot is checked in the stability experiments. On customers request we can prepare the cTnI calibrators with antigen concentrations you need.

Ordering information

Product	Cat. #	Purity	Source
Troponin I Cardiac Calibrator Set	**8T60**		Suggested Range from 0 ng/ml to 100 ng/ml

1.4. cTnI free serum

Prepared from: *pooled normal human serum*
Method of purification: *immunoaffinity chromatography*
Delivery form: *frozen liquid*

cTnI free serum is prepared from pooled normal human serum by immunoaffinity chromatography method. The affinity sorbent utilizes several MAbs with different epitope specificity - to eliminate from serum not only intact cTnI molecule, but also its proteolytical fragments.

cTnI free serum can be used as a matrix for standard and calibrator preparations.

Ordering information

Product	Cat. #
Cardiac Troponin I Free serum	**8TFS**

1.5. Human skeletal troponin I (skTnI)

Source: *human skeletal tissue*
Purity: *95 %*
Composition: *slow and fast skTnI*
Presentation: *lyophilized*
Application: *immunogen for antibody production, calibrators and standard preparation, troponin biochemical and immunochemical studies*
Remarks: *cTnI has poor solubility in buffers with physiological salt concentrations and pH values*

Skeletal isoforms of troponin I (skTnI) were suggested to be used as markers of acute and chronic skeletal muscle injuries. In skeletal muscles troponin I presented by two forms – slow skeletal (186 amino acid residues, 21561 MW, theoretical pI: 9.61) and fast skeletal (181 amino acid residues, 21207 MW, theoretical pI: 8.88).

skTnI is purified from human skeletal tissue by immunoaffinity method followed by additional ion-exchange chromatography. On SDS-PAGE it is presented by two bands.

Ordering information

Product	Cat. #	Purity	Source
Troponin I Skeletal Muscle, Human	**8T25**	>95 %	Human Skeletal Muscle

2. Troponin T

2.1. Human cardiac troponin T (cTnT)

Source: *human cardiac tissue*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *immunogen for the antibody production, mass cTnT standard, troponin biochemical and immunochemical studies*
Handling: *reconstitute in deionized water*

In addition to cTnI, cardiac isoform of TnT is also widely used as a marker of myocardial cell death. cTnT as a marker has the same kinetics of release into patients blood stream and the same sensitivity for minor myocardial events as cTnI, but is considered to be less cardiac specific.

cTnT is purified from human cardiac tissue by immunoaffinity method followed by additional ion-exchange chromatography and is presented by a single band on SDS-PAGE with apparent molecular weight 39 kDa (Fig. 1. Lane 4).

Ordering information

Product	Cat. #	Purity	Source
Troponin T Cardiac, Human	**8T13**	>95 %	Human Cardiac Muscle

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2.2. Human skeletal troponin T (skTnT)

Source: *human skeletal tissue*
Purity: *≥ 95 %*
Composition: *slow and fast skTnT*
Presentation: *lyophilized*
Application: *immunogen for antibody production, troponin biochemical and immunochemical studies*

Two isoforms of TnT are expressed in human skeletal muscles. One – typical for slow skeletal muscle (277 amino acid residues, 32817 MW, theoretical pI: 5.86), another for fast skeletal muscle (257 amino acid residues, 30465 MW, theoretical pI: 6.07).

Isolated human skeletal troponin T consists of both isoforms and on SDS-PAGE is presented by two bands.

Ordering information

Product	Cat. #	Purity	Source
Troponin T Skeletal Muscle, Human	**8T24**	>95 %	Human Skeletal Muscle

3. Troponin C

3.1. Troponin C from human cardiac tissue

Source: *human cardiac tissue*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *immunogen for antibody production, stabilizer of TnI in solutions, troponin biochemical and immunochemical studies*
Handling: *reconstitute in deionized water*

Two forms of troponin C (TnC) are expressed in human muscles. One is typical for slow skeletal muscles, – another – for fast skeletal muscles. In myocardium TnC is presented by a slow skeletal isoform consisting of 161 amino acid residues, 18416 molecular weight and theoretical pI=4.05.

TnC forms high affinity complexes with cTnI. It was demonstrated that in blood stream of AMI patients cTnI is presented mainly as a complex with TnC.

In binary cTnI-TnC complex TnC protects cTnI from protease cleavage so TnC can be used as a natural stabilizer of cTnI in water solutions.

TnC is purified from human cardiac tissue by immunoaffinity method followed by additional ion-exchange chromatography. On SDS-PAGE TnC is presented by a single band with apparent molecular weight 18 kDa (Fig. 1, Lane 2).

Ordering information

Product	Cat. #	Purity	Source
Troponin C	**8T57**	>95 %	Human Cardiac Muscle

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4. Troponin antigens from different animal species

Troponin complex, isolated cardiac and skeletal troponins T, I and C from other animal species can be prepared on customer request.

Ordering information

Product	Cat. #	Purity	Source
Troponin Complex (I-T-C), Mouse	**8T62m**	N/A	Mouse Cardiac Muscle
Troponin Complex (I-T-C), Rat	**8T62r**	N/A	Rat Cardiac Muscle
Troponin Complex (I-T-C), Canine	**8T62c**	N/A	Canine Cardiac Muscle
Troponin Complex (I-T-C), Bovine	**8T62b**	N/A	Bovine Cardiac Muscle
Troponin Complex (I-T-C), Porcine	**8T62p**	N/A	Porcine Cardiac Muscle
Troponin I Cardiac, Mouse	**8T53m**	>95 %	Mouse Cardiac Muscle
Troponin I Cardiac, Rat	**8T53r**	>95 %	Rat Cardiac Muscle
Troponin I Cardiac, Canine	**8T53c**	>95 %	Canine Cardiac Muscle
Troponin I Cardiac, Bovine	**8T53b**	>95 %	Bovine Cardiac Muscle
Troponin I Cardiac, Porcine	**8T53p**	>95 %	Porcine Cardiac Muscle
Troponin I Skeletal Muscle, Mouse	**8T25m**	>95 %	Mouse Skeletal Muscle
Troponin I Skeletal Muscle, Rat	**8T25r**	>95 %	Rat Skeletal Muscle
Troponin I Skeletal Muscle, Canine	**8T25c**	>95 %	Canine Skeletal Muscle
Troponin I Skeletal Muscle, Bovine	**8T25b**	>95 %	Bovine Skeletal Muscle
Troponin I Skeletal Muscle, Porcine	**8T25p**	>95 %	Porcine Skeletal Muscle
Troponin T Cardiac, Mouse	**8T13m**	>98 %	Mouse Cardiac Muscle
Troponin T Cardiac, Rat	**8T13r**	>98 %	Rat Cardiac Muscle
Troponin T Cardiac, Canine	**8T13c**	>98 %	Canine Cardiac Muscle
Troponin T Cardiac, Bovine	**8T13b**	>98 %	Bovine Cardiac Muscle
Troponin T Cardiac, Porcine	**8T13p**	>98 %	Porcine Cardiac Muscle
Troponin T Skeletal Muscle, Mouse	**8T24m**	>95 %	Mouse Skeletal Muscle
Troponin T Skeletal Muscle, Rat	**8T24r**	>95 %	Rat Skeletal Muscle
Troponin T Skeletal Muscle, Canine	**8T24c**	>95 %	Canine Skeletal Muscle
Troponin T Skeletal Muscle, Bovine	**8T24b**	>95 %	Bovine Skeletal Muscle
Troponin T Skeletal Muscle, Porcine	**8T24p**	>95 %	Porcine Skeletal Muscle
Troponin C, Mouse	**8T57m**	> 95 %	Mouse Cardiac Muscle
Troponin C, Rat	**8T57r**	> 95 %	Rat Cardiac Muscle
Troponin C, Canine	**8T57c**	> 95 %	Canine Cardiac Muscle
Troponin C, Bovine	**8T57b**	> 95 %	Bovine Cardiac Muscle
Troponin C, Porcine	**8T57p**	> 95 %	Porcine Cardiac Muscle

5. Troponin-coupled matrixes

All isolated components of troponin complex could be coupled to the different matrixes (Agarose, Sepharose, others) on customers request.

II Troponin-specific antibodies

1. Anti-cTnI monoclonal antibodies

Host animal:	*mice BALB/c*
Cell line used for fusion:	*Sp2/0*
Antigen:	*purified (free) cTnI or cTnI in ternary troponin complex*
Specificity:	*specific to human cTnI (no cross-reaction with skeletal forms of TnI or cTnT and TnC)*
Epitope specificity:	*determined*
Purification method:	*protein-A affinity chromatography*
Presentation:	*MAb solution in PBS with 0.1 % sodium azide*

Hybridomas, producing MAbs were generated after immunization of Balb/c mice with isolated (”free”) cTnI or with cTnI in the form of native troponin complex. MAbs were tested to have no cross-reaction with skeletal forms of the protein as well as to have no cross-reaction with cTnT and TnC. All antibodies are checked on their ability to recognize complexed and free forms of the protein. Some of them are specific to both (complexed and free forms) of cTnI - some in different degree affected by complex formation. MAb 414 recognizes only free form of the molecule. MAbs 6F9 and C5 recognizes both - cardiac and skeletal forms of human TnI.

The epitope specificity of all MAbs was precisely determined by SPOT technique (Fig. 3).

Recently it was demonstrated that during the incubation in the necrotic muscle after AMI, cTnI is cleaved by endogenous proteases. As a result - mixture of intact cTnI molecule and its proteolytic fragments can be detected in the blood stream several hours after onset of the chest pain. Different parts of cTnI molecule display different stability. The most stable is the fragment, located between 30 and 110 amino acid residues, possibly because of its protection by TnC. So far, for better sensitivity and reproducibility in assay design we recommend to use antibodies that recognize the stable part of the molecule and are not affected by cTnI-TnC complex formation.

In Table 2 you can find the data demonstrating the crossreactivity of MAbs with cTnI from several animal species. As it follows from the Table majority of presented monoclonal antibodies can be used for the immunodetection of cTnI from different other animal species. Such MAbs can be useful for scientists working with cTnI of non-human origin.

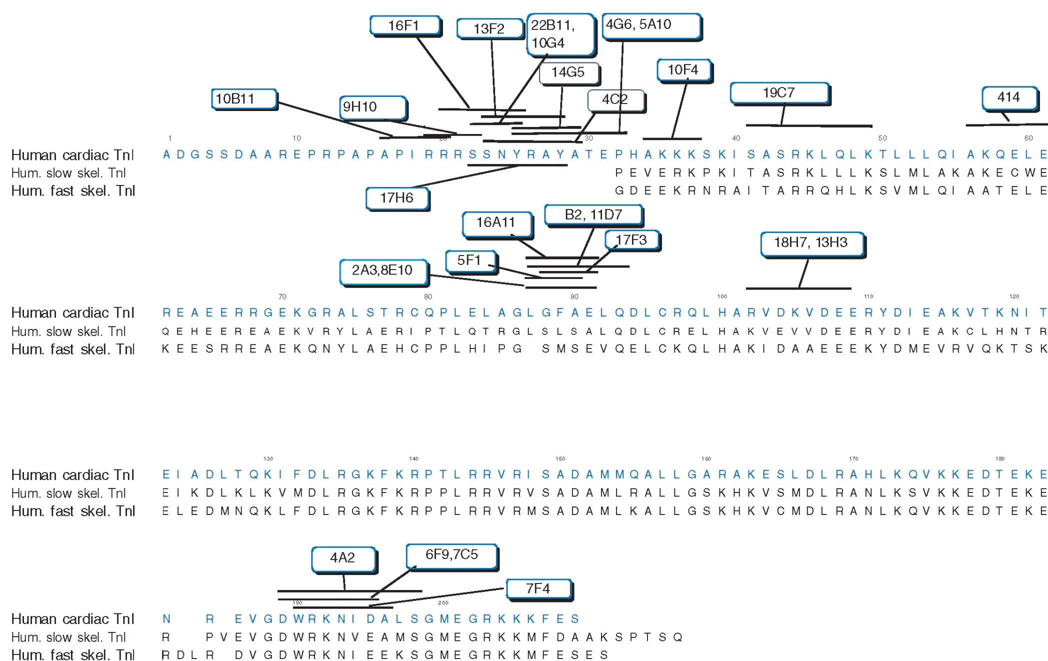


Fig. 3 Epitope mapping of anti-cTnI monoclonal antibodies

Clone	Subclass	Epitope	Cross-reaction (in Western blotting)									
			Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
10B11	IgG1	16-APIRR-20	+	-	+	-	+	+	+	+	-	-
16F1	IgG2b	20 - RRSSN - 24	++	-	+	-	-	-	-	-	-	-
22B11	IgG2b	20 - RRSSN - 24	++	-	+	-	-	-	-	-	-	-
4C2	IgG2a	23 - SNYRAYA - 29	++	++	++	++	++	++	+	++	++	-
14G5	IgG1	25 - YRAYA - 29	+	+	+	+	+	+	+	+	+	-
4G6	IgG2a	25 - YRAYATEP - 32	++	++	++	++	++	++	++	++	++	-
10F4	IgG2a	34 - AKKK - 37	++	++	++	++	++	++	++	++	+	-
19C7	IgG2b	41 - SASRKLQLK - 49	++	++	+	++	+	++	++	++	+	++
414	IgG1	56 - AKQELE - 61	+	+	+	-	+	+	-	-	+	+
8E10	IgG1	87 - LGFAE - 91	+	+	+	+	+	+	+	-	-	-
16A11	IgG1	87 - LGFAE - 91	+	+	+	+	+	+	+	-	-	-
17F3	IgG1	88 - GFAE - 91	+	+	-	+	+	+	+	-	-	-
18H7	IgG1	102 - RVDKVDE - 108	+	+	+	+	+	+	+	+	+	-
6F9	IgG2a	189 - DWRKNID - 195	++	++	++	++	++	++	++	++	++	+
4A2	IgG1	189 - DWRKNIDALS - 198	+	+	+	+	+	+	+	+	+	-
7F4	IgG1	190 - WRKNIDA - 196	+	+	+	+	+	-	+	+	+	-
3C7 **New clone**	IgG1	25 - 40 aar (competition binding data)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23C6 **New clone**	IgG2a	15 - 25 aar (competition binding data)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23D10 **New clone**	IgG2b	85 - 95 aar (competition binding data)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 2. Anti-cTnI Monoclonal Antibodies

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Troponin I Cardiac	**4T21**	4C2	IgG2a	23-29 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	19C11	IgG1	20-23 a.a.r EIA, WB
Troponin I Cardiac	**4T21**	14G5	IgG1	25-29 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	19C7	IgG2b	41-49 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	16A11	IgG1	87-91 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	17F3	IgG1	88-91 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	18H7	IgG1	102-108 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	8E10	IgG1	87-91 a.a.r EIA, WB
Troponin I Cardiac	**4T21**	10F4	IgG2a	34-37 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	7F4	IgG1	190-196 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	10B11	IgG1	16-20 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	2A3	IgG1	87-91 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	5F1	IgG2b	87-90 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	11D7	IgG1	87-93 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	414	IgG1	56-61 a.a.r. EIA, WB
Troponin I Cardiac **New clone**	**4T21**	3C7	IgG1	25-40 a.a.r. EIA, WB
Troponin I Cardiac **New clone**	**4T21**	23D10	IgG2b	85-95 a.a.r. EIA, WB
Troponin I Cardiac **New clone**	**4T21**	23C6	IgG2a	15-25 a.a.r. EIA, WB
Troponin I Cardiac	**4T21**	B2	IgG2b	87-93 a.a.r. EIA, WB
				3 % C/R skeletal troponin I
Troponin I Cardiac	**4T21**	6F9	IgG2a	187-193 a.a.r. EIA, WB
				100 % C/R skeletal troponin I
Troponin I Cardiac	**4T21**	C5	IgG1	EIA, WB, 5 % C/R
				skeletal troponin I

1.1. Antibody application

1.1.1. cTnI quantitative sandwich immunoassay

All cTnI specific MAbs were tested in sandwich fluoroimmunoassay as capture and detection antibodies. The best pairs (capture – conjugate):

Capture MAb	Conjugate MAb
8E10	19C7
19C7	8E10
19C7	16A11
16A11	19C7
18H7	16A11

demonstrated high sensitivity (0.02 ng/ml and better, Fig. 4), good kinetics, low background and high reproducibility. Immunoassays, based on these antibodies, recognize complexed and free forms of the antigen with the same sensitivity. Epitopes of these antibodies are located in the central (most stable) part of troponin I molecule and are not affected by phosphorylation or presence of heparin in the sample.

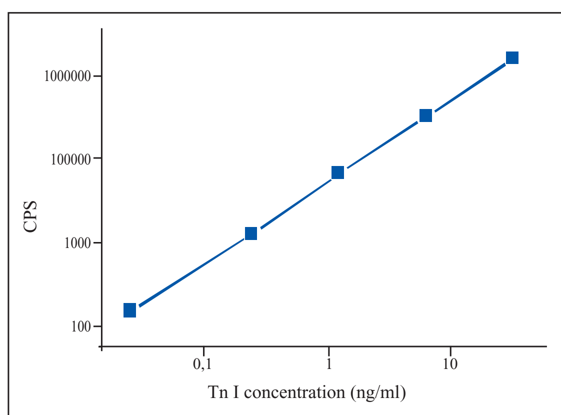


Fig. 4. cTnI calibration curve:
One step assay in streptavidin coated plates
Monoclonal antibodies:
- capture: biotinylated 19C7; 200 ng/well
- detection: Eu-labeled 8E10; 200 ng/well
Sample volume: 50 µl
Antigen: cTnI (native troponin complex)
Incubation time: 20 min
Temperature: 20°C

Advantages:
- MAbs with high affinity
- high sensitivity and kinetics of the assay
- all recommended MAbs combinations checked with AMI serum sample
- MAbs recognizing stable part of the molecule
- are not affected by cTnI - TnC complex formation
- are not affected by heparin
- are not affected by phosphorylation of the antigen with protein kinase A

1.1.2. cTnI qualitative assay (quick bedside tests)

For the development of qualitative assays we are suggesting to use one MAb with the epitope located in the central stable part of the molecule as a capture (MAbs 19C7 or 16A11) and one or combination of several high affinity MAbs, recognizing different parts of the molecule (16A11, 19C7, 4C2, 14G5 and 7F4) as detection (Table 3). Good results (high sensitivity, low background) were achieved with new MAb 3C7 generated recently and polyclonal anti-cTnI antibodies (see below).

Capture		Detection
19C7		16A11 or 16A11+7F4+4C2
16A11		19C7 or 19C7+7F4+4C2
3C7	**New clone**	polyclonal antibodies
3C7	**New clone**	19C7
23D10	**New clone**	19C7
23C6	**New clone**	19C7

TABLE 3. Possible combinations of MAbs for qualitative assay

Advantages:
- high sensitivity
- low background

1.1.3. Immunoassays for the detection of cTnI from other species

In clinical studies of new drugs, new methods of surgery done on experimental animals the effect of new technology on cardiac function and on cardiac myocyte viability can be important. As it follows from Table 2 the majority of MAbs have wide specificity and recognize not only human cTnI but also cardiac troponin antigens from other species. Immunoassays utilising these antibodies could be used for the quantitative measurement of cTnI in the blood of different animals. Two two-site combinations of antibodies – 4C2-19C7 and 10F4-19C7 were selected for high sensitivity, good reproducibility and reactivity with wide range of the cardiac troponin I antigens from different animal species. Both combinations were tested with cTnI purified from bovine, rabbit, rat and mouse heart tissues. Taking into consideration that MAbs 4C2 (epitope 23-SNYRAYA-29 for human cTnI)- and 10F4 (epitope 34-AKKK-37) recognize all tested antigens except cTnI from fish cardiac tissue, whereas MAb 19C7 (epitope 41-SASRKLQLK-49) cross-react with all tested cardiac troponin I antigens, we can state that both assays could be used for quantitative immunodetection of cTnI from wide variety of animal species. Fig. 5A demonstrates performance of antibodies in Western blotting with rat cTnI. On Fig. 5B the calibration curve for the assay of rat cTnI utilizing MAb 4C2 as a capture and MAb 19C7 as a detection is presented.

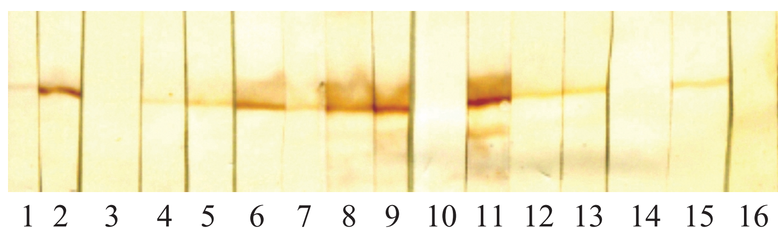


Fig. 5A. Detection of cTnI (rat) by different monoclonal antibodies.
1 – B2; 2 – C5; 3 – 2A3; 4 – 3C4; 5 – 4A2; 6 – 4C2; 7 – 4C5; 8 – 4G6; 9- 5A10; 10 – 5F1; 11 – 6F9; 12 – 7C5; 13 – 7F4; 14 – 8E10; 15 – 9H7; 16 – 9H10.

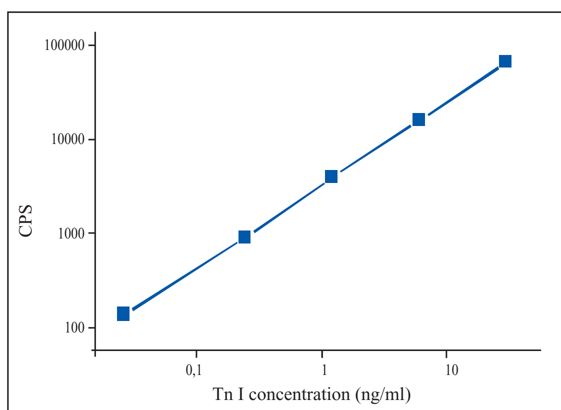


Fig. 5B. Assay 4C2 – 19C7. Calibration curve for purified rat cardiac TnI. Monoclonal antibodies:
- capture: biotinylated 4C2; 200 ng/well
- detection: Eu-labeled 19C7; 200 ng/well

Sample volume:	*50 µl*
Antigen:	*rat cardiac TnI*
Incubation time:	*20 min*
Temperature:	*20°C*

1.1.4. Detection of free cTnI molecule (not in the complex with TnC)

MAb 414 interacts only with the free form of cTnI and does not recognize cTnI in complex with TnC. The epitope of this MAb is closed or changed by TnC in binary TnI - TnC complex. Thus this monoclonal antibody can be used only for the immunodetection of free form of the antigen. For quantitative immunodetection of the free cTnI we suggest to use 414 MAb as detection and monoclonal antibodies 7F4 or 10F4, or 8E10 as capture (Fig. 6).

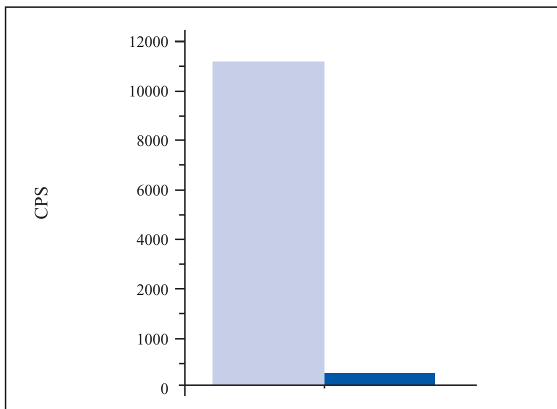
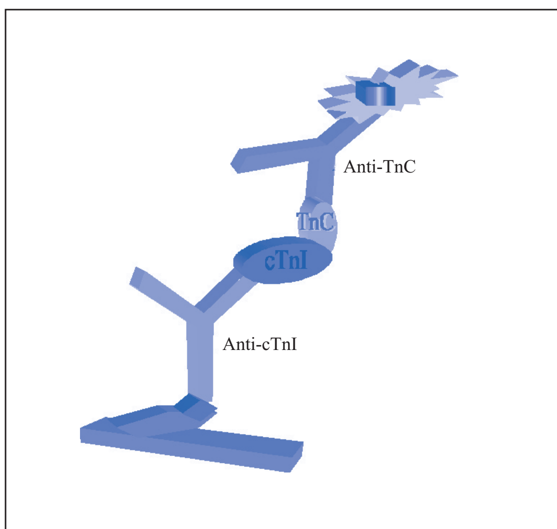


Fig. 6. Detection of free cTnI
Two forms of cTnI detected by sandwich immunoassay with 10F4 MAbs used as capture and 414 a detection MAb
Blue column - free cTnI (30 ng/ml)
Dark blue column - cTnI (30 ng/ml) in binary cTnI - TnC complex

1.1.5. Detection of binary cTnI-TnC complex

For the quantitative measurements of the binary cTnI - TnC complex we suggest to use the principle of a "mixed" sandwich immunoassay (Fig. 7 A, B). In such assay the detection MAb is cTnI specific (19C7) whereas capture antibody (7B9) recognizes TnC. Though the main part of cTnI in the blood stream of AMI patients is presented in the form of the complex with TnC, results of cTnI measurements by this assay in AMI serum are in good correlation with results received by cTnI assay (Fig. 7C).

A.



B.

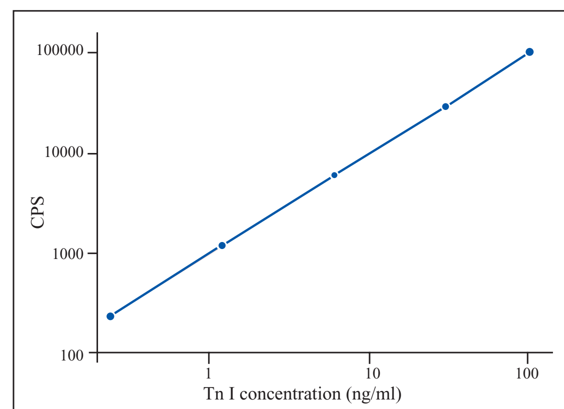
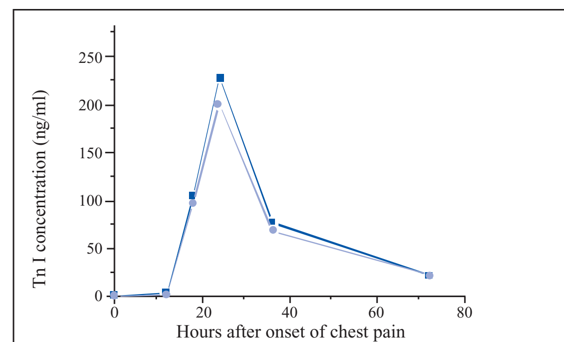


Fig. 7. Measurement of cTnI-TnC complex by mixed assay
A. Scheme of the assay
B. Calibration curve (ternary native complex as a standard)
C. cTnI concentration in the form of cTnI-TnC complex (circles; measured by mixed assay) and total cTnI concentration (free and complexed - square) measured in AMI serum

C.



1.1.6. Detection of binary cTnI-cTnT complex

The same approach as described in previous paragraph can be used for the quantitative immunodetection of the binary cTnI-cTnT complex. We suggest to use anti-cTnT MAb 1F2 as capture and anti-cTnI MAb 19C7 as a detection. Such assay has no cross-reaction with free forms of cTnI, cTnT or TnC, but recognizes only cTnI-cTnT binary or cTnI-cTnT-TnC ternary complex.

1.1.7. Detection of dephosphorylated cTnI by Western blotting or by sandwich immunoassay

cTnI is phosphorylated *in vivo* and can be phosphorylated *in vitro* by cAMP-dependent protein kinase A. Sites of phosphorylation are the serines in the 22nd and 23rd position. Some of HyTest's MAbs recognize the epitopes containing these two amino acid residues. It was demonstrated recently that one of these MAbs – namely MAb 22B11 recognizes only dephosphoform of cTnI and does not interact with mono- or biphosphorylated antigen (Fig. 8). 22B11 was suggested to be used for qualitative or semi quantitative immunodetection of dephosphorylated cTnI in Western blotting or for quantitative measurements of dephosphorylated cTnI in sandwich immunoassay (Fig. 9). Using such immunoassay we recently demonstrated that significant part of cTnI in patient's blood is partially phosphorylated (Fig. 10).

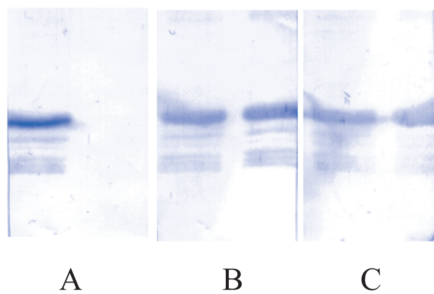


Fig. 8. Monoclonal antibodies 22B11(A), 10B11(B) and 8E10 (C), recognizing dephospho- and phospho forms of human cardiac TnI.

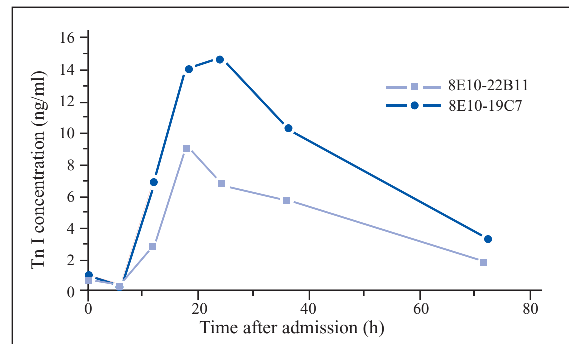


Fig.10. cTnI measurements: total (circles) and dephosphorylated (squares) CTnI in serial blood samples from AMI patient.

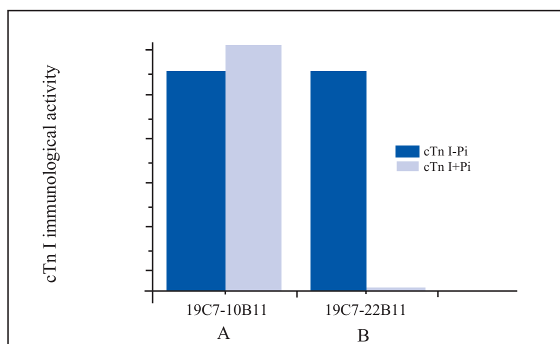


Fig. 9. Two immunoassays recognizing dephospho-(dark blue columns) and biphospho-(light blue columns) forms of cTnI.

1.1.8. Detection of cTnI or cTnI fragments by Western Blotting and immunofluorescent staining

All MAbs recognize human cTnI or cTnI fragments in Western blotting (Fig 11A). But for better sensitivity in Western blotting we recommend to use 4G6, 19C7, 16A11, 6F9 and 7F4 MAbs.

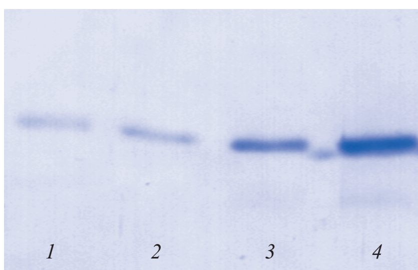


Fig. 11A. Detection of cTnI by Western blotting
After SDS-PAGE cTnI was transferred on nitrocellulose membrane and probed by 16A11 MAbs and then by goat anti-mouse IgG antibodies, conjugated with HRP. Substrate - diaminobenzidine.
Lane 1 - 3 ng cTnI
Lane 2 - 10 ng cTnI
Lane 3 - 30 ng cTnI
Lane 4 - 100 ng cTnI

Some of monoclonal antibodies presented in the Table 2 were tested for immunofluorescent staining of TnI in tissue sections and cell cultures. Good results were obtained with MAb 6F9 which can be used for immunodetection of both - cardiac and skeletal Troponin I antigens from wide variety of animal species (Fig 11B).

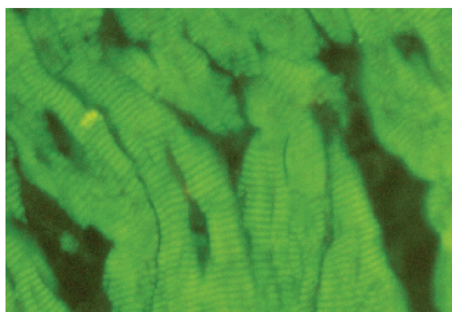


Fig 11B. Bovine cardiac tissue. Immunofluorescent staining for TnI using MAb 6F9.

2. Anti-cTnI polyclonal antibodies

Host animal:	*rabbit*
Antigen:	*isolated (free) cTnI or cTnI in ternary troponin complex*
Specificity:	*specific to human cTnI (on request: polyclonal antibodies with defined epitope specificity)*
Purification method:	*immunoaffinity chromatography*

To generate anti-cTnI polyclonal antibodies we are using two protocols of animal immunization – with purified protein and with cTnI in the form of native troponin complex. Polyclonal antibodies are purified by immunoaffinity method utilizing cTnI-Sepharose CL4B as an affinity matrix.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Troponin I Polyclonal	**4T21/2**	N/A	N/A	Host animal - rabbit

HyTest

3. Anti-cTnT monoclonal antibodies

Host animal:	*mice BALB/c*
Cell line used for fusion:	*Sp2/0*
Antigen:	*human or bovine cTnT*
Epitope specificity:	*partially determined*
Purification method:	*protein-A affinity chromatography*
Presentation:	*MAb solution in PBS with 0.1 % sodium azide*

Hybridomas, producing MAbs were generated after immunization of Balb/c mice with human or bovine cTnI. MAbs were tested to have no cross-reaction with cTnI and TnC.

The epitope specificity of some MAbs was precisely determined by SPOT technique. For MAbs not interacting with SPOT peptides epitopes were localized using cTnT BrCN peptides.

Crossreaction of anti-human cTnT MAbs with cTnTs from other species was studied in Western blotting with crude extracts of animal's cardiac tissue (Table 4).

Clone	Subclass	Epitope	Cross-reaction (in Western blotting)									
			Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
1F2	IgG1	peptide 60-70	+	+	-	+	+	+	+	-	-	+
2D10	IgG1	peptide 60-70	+	-	-	-	+	-	-	-	-	-
2EG11	IgG2a	66-75	+	+	+	+	+	+	+	-	+	-
7F4	IgG2b	61-70	++		++		-	-	-			-
7G7	IgG1	peptide 60-70	+	+	-	-	-	-	-	-	-	-
3D6	IgG1	70-94	+	+	-	+	+	+	-	-	+	+
2F3	IgG2b	-	++	+	++	++	+	+	+	+	+	+
2G3	IgG2b	peptide 94-180	++	+	+	+	+	+	+	+	+	-
6G9	IgG1	peptide 94-180	+	+	-	+	+	+	-	+	+	+
1F11	IgG2b	146-160	++	++	++	++	+	+	+	+	+	+
1A11	IgG2b	146-160	++	++	++	++	+	+	+	+	++	+
4D11	IgG2b	146-160	++	++	++	++	+	+	+	+	++	++
7A9	IgG1	peptide 180-287	+	+	-	+	+	+	-	-	-	-

Table 4. Anti-cTnT monoclonal antibodies

Ordering information

Product	Cat. #	Clone	Subclass	Remarks	
Troponin T Cardiac	**4T19**	5C12	IgM	180-258 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	1F2	IgG1	60-71 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	2F3	IgG2b	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	7G7	IgG1	60-71 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	6G9	IgG1	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	1A11	IgG2b	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	9G6	IgG1	1-60 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	9G8	IgG1	71-95 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	21G1	IgG2b	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	1C11	IgG1	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	2G3	IgG2b	95-181 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	1F11	IgG2b	146-160 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	7F4	IgG2b	60-70 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	7A9	IgG1	180-287 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	2D10	IgG1	60-70 a.a.r.	EIA, WB
Troponin T Cardiac	**4T19**	3D6	IgG1	71-95 a.a.r.	EIA, WB

HyTest

4. Anti-cTnT polyclonal antibodies

Host animal: *rabbit*
Antigen: *human cardiac troponin complex or human cTnT*
Purification method: *affinity chromatography (cTnT-agarose)*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*

Animals are immunized with purified protein or with cTnT in the form of native troponin complex. Antibodies are purified by immunoaffinity method utilizing cTnT agarose as an affinity matrix.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Troponin T Polyclonal	**4T19/2**	N/A	N/A	Host animal – rabbit

5. Anti-cTnC monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human cardiac troponin complex or isolated slow skeletal cardiac isoform of TnC*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*

Hybridomas, producing MAbs were generated after immunization of Balb/c mice with isolated slow skeletal ("cardiac") form of TnC or by TnC in the form of native troponin complex. MAbs were tested to have no cross-reaction with cTnT and cTnI. All MAbs recognize TnC in binary complex with cTnI.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Troponin C Cardiac	**4T27**	1A2	IgG2a	Capture, EIA, WB
Troponin C Cardiac	**4T27**	7B9	IgG1	Conjugate, EIA, WB
Troponin C Cardiac	**4T27**	12G3	IgG2b	EIA, WB

6. Anti-skeletal Troponin I monoclonal antibodies

Recent studies revealed that skeletal isoforms of troponin I (skTnI) could be used as specific and sensitive markers of skeletal muscle injury.

Monoclonal antibodies were generated after mice immunization with troponin I purified from human skeletal tissue. Specificity of antibodies was confirmed in ELISA and Western blotting (Fig. 11C). According to the Western blotting analysis all antibodies recognized both – slow and fast skeletal isoforms of skTnI. Competition studies revealed that new antibodies were specific to three epitopes on the molecule's surface.

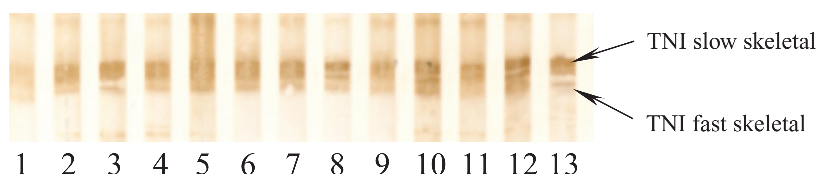


Fig 11C. Detection of TnI (slow and fast skeletal) by different monoclonal antibodies in Western Blotting.
1 – 1A10; 2 – 2G12; 3 – 2H9; 4 – 3H7; 5 – 3H10; 6 – 6F11; 7 – 7G2; 8 – 7H6; 9- 8B9; 10 – 8D12; 11 – 11E6; 12 – 11G8; 13 – 12F10.

The best sensitivity (0.3 ng/ml) with skTnI, purified from human skeletal muscle was achieved in the assays utilizing MAbs 12F10 - 7G2 and C5 - 8B9 (Fig.11D). Assays had no detectable cross-reaction with the cardiac isoform of the antigen (up to 300 ng/ml). Preliminary clinical studies demonstrated that both assays recognized the antigen in the blood of patients with chronic muscle disease and could be used for the determination of skTnI in human blood.

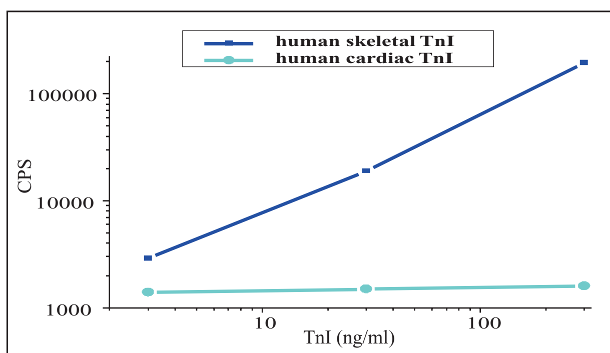


Fig. 11D. Detection of skeletal (-▪-) and cardiac (-●-) isoforms of human troponin by C5 – 8B9 immunoassay.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Troponin I Skeletal Muscle	**4T20**	2H9	IgG2b	Capture, EIA, WB
Troponin I Skeletal Muscle	**4T20**	12F10	IgG2b	Capture, EIA, WB
Troponin I Skeletal Muscle	**4T20**	7G2	IgG2b	Conjugate,WB, EIA
Troponin I Skeletal Muscle	**4T20**	6F11	IgG2a	Conjugate,WB, EIA
Troponin I Skeletal Muscle	**4T20**	8B9	IgG2a	Conjugate,WB, EIA
Troponin I Skeletal Muscle	**4T20**	11E6	IgG2a	Conjugate,WB, EIA

7. Immunosorbents

All anti-TnT, TnI or TnC antibodies – monoclonal as well as polyclonal - can be coupled to different types of matrixes (Agarose, Sepharose, other) on customer’s request.

Product	Cat. #	Remarks
Affinity matrix with anti-TnI (TnT or TnC) antibodies	**OEM**	Indicate matrix (Agarose, Sepharose, other) and clone name

8. Labeled antibodies

Conjugates of anti-TnT (TnI or TnC) antibodies with FITC, Texas Red, Biotin, HRP can be prepared on customers request.

Product	Cat. #	Remarks
Conjugate of anti-TnI (TnT or TnC) antibodies	**OEM**	Indicate label matrix (FITC, Texas Red, Biotin, HRP) and clone name

HyTest

III Myoglobin

1. Human myoglobin

Source: *human cardiac tissue*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *immunogen for antibody production, immunological and mass myoglobin standard, myoglobin biochemical and immunochemical studies*
Handling: *reconstitute in deionized water.*

Myoglobin is a small hem-containing protein (153 amino acid residues, molecular weight (w/o heme) - 17053 Da and theoretical pI=7.29) responsible for the oxygen deposition in muscle tissue. One and the same form of myoglobin is expressed in cardiac and skeletal muscles. No cardiac-specific form is known for myoglobin.

As a marker of myocardial damage myoglobin is known and used for more than three decades and now is still very common in clinical practice as an early marker of AMI. It appears in patients blood 1 – 3 hours after onset of the symptoms, reaching peak level within 8 – 12 hours. Myoglobin is not so cardiac specific as cTnI or cTnT. Because of high myoglobin concentration in skeletal muscle tissue, even minor skeletal muscle injury results in the significant increase of myoglobin's concentration in blood. Because of this for better specificity in AMI diagnosis myoglobin is used together with cTnI or cTnT.

Myoglobin is purified from human cardiac tissue by several chromatographic steps including gel-filtration and anion-exchange chromatography. After SDS-PAGE in reducing conditions myoglobin is presented by a single band with apparent molecular mass 17 kDa.

Product	Cat. #	Purity	Source
Myoglobin	**8M50**	>98 %	Human Cardiac Muscle

2. Anti-myoglobin monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human myoglobin*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*

Hybridomas, producing MAbs were generated after immunization of Balb/c mice with purified human myoglobin. Different combinations of monoclonal antibodies could be used for the immunoassay development. The best combinations utilize 4E2 MAb as capture, 7C3 MAb as a detection or vice versa. Assay gives good results with serum samples from AMI patients.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Myoglobin, Human	**4M23**	4E2	IgG1	Capture, EIA
Myoglobin, Human **New clone**	**4M23**	7C3	IgG2a	Conjugate, EIA
Myoglobin, Human	**4M23**	908	IgG1	Conjugate, EIA

3. Myoglobin free serum

Prepared from: *pooled normal human serum*
Method of purification: *immunoaffinity chromatography*
Delivery form: *frozen liquid*

Myoglobin free serum is prepared from pooled normal human serum by immunoaffinity chromatography. The matrix for affinity sorbent utilizes several monoclonal antibodies with different epitope specificity.

Myoglobin free serum can be used as a matrix for standard and calibrator preparations

Ordering information

Product	Cat. #
Myoglobin Free serum	**8MFS**

IV. Fatty Acid Binding Protein (FABP)

1. Human Fatty Acid Binding Protein

Source: *human cardiac tissue*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *immunogen for antibody production, immunological and mass FABP standard, FABP biochemical and immunochemical studies*
Handling: *reconstitute in deionized water*

Fatty Acid Binding Protein (FABP) is a small cytosolic protein responsible for the transport and deposition of fatty acids inside the cell. Several different isoforms of FABP are expressed in different tissues types. Cardiac isoform of FABP (cFABP) is expressed mainly in cardiac muscle tissue and in significantly lower concentration in skeletal muscles. Cardiac isoform of FABP (cFABP) consists of 132 amino acid residues, molecular weight – 14727 Da and theoretical pI=6,34.

Recently it was demonstrated that cFABP can be used as an early marker of myocardial infarction. cFABP has the same kinetics of liberation into patients blood as myoglobin. But because of significantly lower cFABP concentration in skeletal muscle (compared to myoglobin) the concentration of cFABP in the blood of healthy donors is also significantly lower (6-10 ng/ml for cFABP and 40 – 60 ng/ml for myoglobin). This makes cFABP more sensitive and reliable early marker of myocardial cell death. Recent studies demonstrated that FABP can be useful also for the early detection minor myocardial events such as unstable angina. Switching clinical studies from myoglobin to cFABP can be helpful in improving early AMI diagnosis.

cFABP is purified from human cardiac tissue by several chromatographic methods including gel-filtration and ion-exchange chromatography. After SDS-PAGE in reducing conditions cFABP is presented as a single band with apparent molecular weight 15 kDa.

Ordering information

Product	Cat. #	Purity	Source
Fatty Acid Binding Protein (FABP)	**8F65**	>98 %	Human Heart Tissue

2. Anti-cFABP monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human cFABP*
Epitope specificity: *partially determined*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*

Four hybridoma cell lines, producing anti-cFABP MAbs were generated after immunization of Balb/c mice with purified human cFABP. Preliminary epitope mapping of monoclonal antibodies is presented in Fig. 12. The best combination for sandwich immunoassay is: 9F3 MAb used as capture antibody and 10E11 as a detection MAb (Fig. 13). The immunoassay, utilizing these monoclonal antibodies was evaluated in clinical studies and demonstrated high sensitivity, good kinetics and good recognition of the antigen in patients samples (Fig. 14).

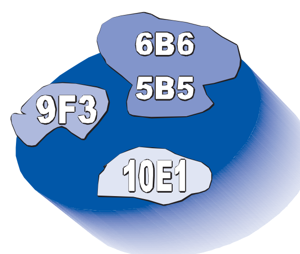


Fig. 12. Epitope mapping of anti-FABP monoclonal antibodies.

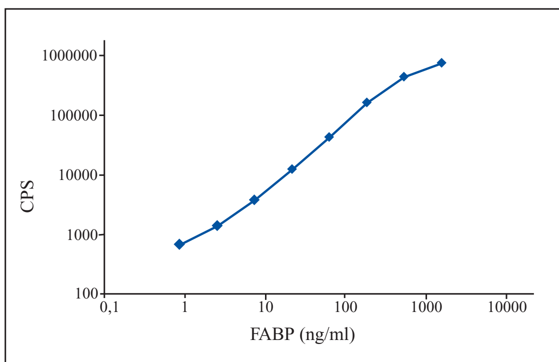


Fig. 13. Calibration curve of FABP sandwich immunoassay. (9F3 – 10E11).

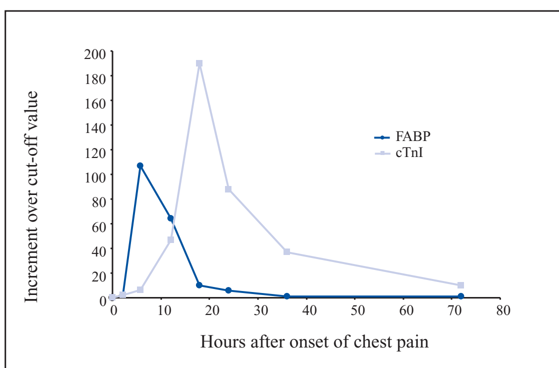


Fig. 14. Time-dependent changes of FABP (dark blue) and cTnI (light blue) concentrations in the blood of AMI patient.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Fatty Acid Binding Protein (FABP)	**4F29**	5B5	IgG1	EIA, Capture, Conjugate
Fatty Acid Binding Protein (FABP)	**4F29**	9F3	IgG1	EIA, Capture, Conjugate
Fatty Acid Binding Protein (FABP)	**4F29**	6B6	IgG2b	FABP Purification by A/C
Fatty Acid Binding Protein (FABP)	**4F29**	10E1	IgG1	EIA, Capture, Conjugate

3. cFABP free serum

Prepared from: *pooled normal human serum*
Method of purification: *immunoaffinity chromatography*
Delivery form: *frozen liquid*

cFABP free serum is prepared from pooled normal human serum by immunoaffinity chromatography. The matrix for affinity sorbent utilizes three monoclonal antibodies with different epitope specificity.

cFABP free serum can be used as a matrix for standard and calibrator preparations.

Ordering information

Product	Cat. #
FABP free serum	**8FFS**

V Glycogen Phosphorylase, BB Isoenzyme (GPBB)

1. Human glycogen phosphorylase, BB isoenzyme (GPBB)

Source: *human cardiac tissue*
Purity: *≥ 95 %*
Presentation: *solution in buffer, containing 1 mM β-glycerophosphate, 1 mM EDTA, 15 mM ME, 0.5 M NaCl, and 50 % glycerol, pH 7.8.*
Application: *immunogen for antibody production, immunological and mass GPBB standard, GPBB, enzymatic, biochemical and immunochemical studies*
Handling: *store at -20°C*

Glycogen phosphorylase BB is an enzyme playing an important role in the glycogen turnover. Three known isoforms of glycogen phosphorylase – GPBB, GPMM and GPLL are expressed in brain and cardiac muscle tissue (GPBB), skeletal muscles (GPMM) and liver (GPLL). GPBB is a homo dimer consisting of two subunits with molecular mass 96682 Da (843 amino acid residues), theoretical pI=6.26.

In 1987 GPBB was for the first time suggested as a marker of acute myocardial ischemia and acute myocardial infarction. GPBB is considered to be an early marker of a myocardial cell death with kinetics of release closely resemble to those of myoglobin and FABP. Recent studies demonstrated that GPBB can be useful in diagnosis of myocardial tissue damage in the patients with bypass surgery, unstable angina and some other cases. All these features make GPBB a very promising marker of myocardial cell injury, but its cardiospecificity still should be determined.

GPBB is purified from human cardiac tissue by several chromatographic methods including gel-filtration and ion-exchange chromatography. After SDS-PAGE in reducing conditions GPBB is presented as a single band with apparent molecular mass 92 kDa. Preparation does not contain more than 2 % of the glycogen phosphorylase MM isoenzyme.

Ordering information

Product	Cat. #	Purity	Source
Glycogen Phosphorylase Isoenzyme BB (GPBB)	**8G67**	>95 %	Human Cardiac Muscle

2. Anti-GPBB monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human GPBB*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *GPBB immunoassay, GPBB immunoaffinity purification, GPBB immunodetection in Western blotting*

Hybridoma clones, producing anti-GPBB MAbs were generated after immunization of Balb/c mice with purified human glycogen phosphorylase. Five MAbs (7B9, 8G7, 6G5, 11C10, and 1G6) are specific for the BB isoenzyme, others recognize MM isoenzyme as well. The best combinations to be used in the assay are:

Capture MAb	Detection MAb
1G6	6F1
1G6	9F5
1G6	17B6
6F1	7B9
17B6	10H5

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
GPBB	**4GP31**	1G6	IgG2b	BB isoenzyme, Capture, EIA
GPBB	**4GP31**	3G1	IgG1	BB and MM isoenzyme, WB
GPBB	**4GP31**	10H5	IgG2a	BB and MM isoenzyme, Conjugate,EIA, WB
GPBB	**4GP31**	10D12	IgG1	BB and MM isoenzyme, WB
GPBB	**4GP31**	17B6	IgG1	BB and MM isoenzyme, Capture, Conjugate, EIA, WB
GPBB	**4GP31**	6F1	IgG1	BB and MM isoenzyme, Conjugate, EIA, WB
GPBB	**4GP31**	7B9	IgG1	BB isoenzyme,Conjugate, EIA, WB
GPBB	**4GP31**	6G5	IgG1	BB isoenzyme, WB
GPBB	**4GP31**	8G7	IgG1	BB isoenzyme, WB
GPBB	**4GP31**	9F5	IgG1	BB and MM isoenzyme, Conjugate, EIA, WB

HyTest

VI C-Reactive Protein (CRP)

1. Human C-reactive protein (CRP)

Source: *human pleural/ascites fluid or plasma*
Purity: *≥ 95 % by SDS-PAGE*
Presentation: *solution in 0.3 M NaCl, 0.05 % sodium azide, 20 mM Tris, pH 8.0*
Application: *immunogen for antibody production, for immunological and mass CRP standard, CRP biochemical and immunochemical studies*
Handling: *store at +4°C*

CRP – "acute phase serum protein" that shares several functions with immunoglobulins, is known for several decades as a non-specific inflammation marker. But among several markers of systemic inflammation, CRP shows the strongest association with vascular events. Recent studies revealed that high-sensitive measurements of C-reactive protein (hsCRP) may provide a new method for detecting patients with high risk of plaque rapture and other myocardial complications. hsCRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women. Addition of hsCRP to standard lipid screening may improve global risk prediction among the individuals with high as well as low cholesterol levels. Available data suggest that hsCRP has the potential to play an important role as an adjunct for global risk assessment in the primary prevention of cardiovascular disease.

C-reactive protein release in acute coronary syndromes may be a response to myocardial necrosis or may reflect the inflammatory process that drives the atherogenesis. Though the inflammatory process has been found to play an important role in the pathogenesis of coronary heart disease, raised concentrations of CRP are predictive of an increased risk of major adverse cardiovascular events for the patients with unstable angina and non-Q wave myocardial infarction, suggesting that the intensity of the vascular inflammatory process at the time of presentation is a determinant of clinical outcome in unstable coronary artery disease. Many clinical studies demonstrated that coronary mortality among the patients with unstable angina and elevated CRP is significantly higher comparing with the patients without elevated CRP, whereas normal baseline serum levels of C-reactive protein identify a subgroup of patients at low risk of cardiac events during follow-up.

At the same time there are hypotheses suggesting possible pathogenetic role of CRP by itself, based on the ability of this protein to bind with low density lipoproteins, to activate complement and on the CRP presence in atheroma and acute myocardial infarction lesions.

Ordering information

Product	Cat. #	Purity	Source
C-Reactive Protein (CRP)	**8C72**	>98 %	Human pleural ascites fluid

2. Anti-CRP monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human CRP*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *CRP immunoassay, CPR immunoaffinity purification*

Hybridoma clones, producing anti-CRP MAbs were generated after immunization of Balb/c mice with purified human CRP. Four MAbs namely C2, C5, C6 and C7 recognize antigen both – in the presence and in the absence (samples containing EDTA) of Ca^{2+}. MAbs C1, C3 and C4 do not interact with $Ca2^{+}$-depleted protein. Best combinations for the development of sandwich immunoassay:

Capture MAb	Detection MAb
C2	C6
C5	C6
C7	C6

Monoclonal antibodies utilized in these two-site combinations interact with the antigen with high affinity and are suitable for the development fast and very sensitive CRP immunoassays. MAbs are insensitive to the presence of Ca^{2+} ions in the sample and can be used for the CRP detection not only in serum, but also in EDTA plasma (Fig. 15).

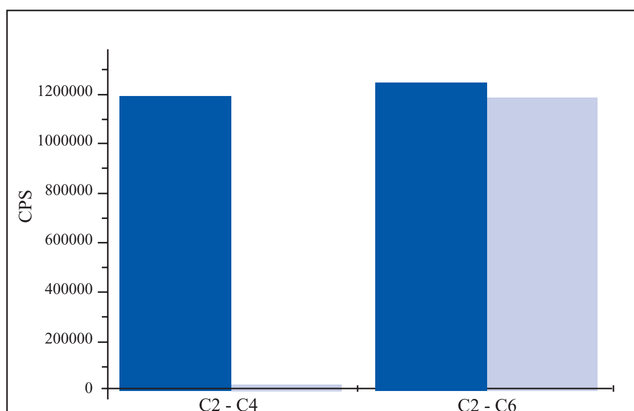


Fig. 15. CRP detection by two two-site combinations of monoclonal antibodies in the presence (2 mM, dark blue columns) and in the absence (5 mM EDTA, light blue columns) of Ca^{2+} ions in the sample. Antibody C4 does not recognize antigen in the absence of Ca^{2+} and as a result – MAb combination C2-C4 does not give any response in the presence of EDTA in the sample. Antigen concentration – 100 ng/ml.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
C-reactive Protein (CRP)	**4C28**	C1	IgG2b	WB
C-reactive Protein (CRP)	**4C28**	C2	IgG1	Capture, Conjugate, EIA
C-reactive Protein (CRP)	**4C28**	C3	IgG1	EIA
C-reactive Protein (CRP)	**4C28**	C4	IgG1	EIA, WB
C-reactive Protein (CRP)	**4C28**	C5	IgG2a	Capture, EIA,
C-reactive Protein (CRP)	**4C28**	C6	IgG2a	Conjugate, EIA,
C-reactive Protein (CRP)	**4C28**	C7	IgG1	Capture, Conjugate, EIA

3. CRP free serum

Prepared from: *pooled normal human serum*
Method of purification: *immunoaffinity chromatography*
Delivery form: *frozen liquid*

CRP free serum is prepared from pooled normal human serum by immunoaffinity chromatography. The matrix for affinity sorbent utilizes three monoclonal antibodies with different epitope specificity.

CRP free serum can be used as a matrix for standard and calibrator preparations.

Ordering information

Product	Cat. #
CRP Free serum	**8CFS**

VII Brain S-100 Protein

1. Human S-100 protein

Source: *human brain*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *immunogen for antibody production, immunological and mass S-100 standard, S-100 biochemical and immunochemical studies*
Handling: *reconstitute in deionized water*

S-100 protein derived from brain tissue is an acidic calcium-binding protein with a molecular weight of about 21 kDa. In brain it is predominantly synthesised by astroglial cells and is mainly presented by two isoforms alpha-beta heterodimer (S-100a) or beta-beta homodimer (S-100b).

Because of its predominant location in glial cells S-100 protein can be used as a sensitive and reliable marker for central nervous system damage. Structural damage of glial cells causes leakage of S-100 protein into the extracellular matrix and into cerebrospinal fluid, further releasing into the bloodstream. S-100 protein appears to be a promising marker for the severity of brain injury and neuronal damage. There is a good correlation between S-100 concentration in patients' serum samples and outcome after traumatic and ischemic brain injury. Measurements of S-100 protein could be very useful in diagnosis and prognosis of clinical outcome in acute stroke and in the estimation of the ischemic brain damage during cardiac surgery. Elevated serum level of S-100 correlates with duration of circulatory arrest.

S-100 protein is purified from human brain tissue by several chromatographic methods including gel-filtration and ion-exchange chromatography. After native gel electrophoresis by Ornstein-Davis S-100 protein is presented by two bands corresponding to alpha-beta and beta-beta forms (Fig. 16).

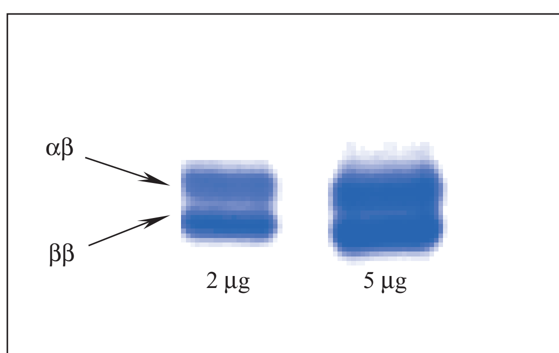


Fig. 16. Native gel electrophoresis of S-100 protein (by Ornstein – Davis)
Gel: *20 % PAGE with 2 mM EGTA*
Antigen loaded: *lane 1 – 2 μg*
lane 2 –5 μg
Gel staining: *Coomassie brilliant blue R-250*

Ordering information

Product	Cat. #	Purity	Source
S-100 Protein	**8S9h**	>95 %	ββ homodimer and αβ heterodimer, Human Brain
S-100 Protein	**8S9b**	>95 %	ββ homodimer and αβ heterodimer, Bovine Brain
S-100 ββ homodimer	**8S92-h**	>95 %	ββ homodimer, Human Brain
S-100 ββ homodimer	**8S92-b**	>95 %	ββ homodimer, Bovine Brain

HyTest

2. Anti-S-100 monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human brain S-100 protein*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *S-100 immunoassay, S-100 immunoaffinity purification, S-100 immunodetection in Western blotting*

Four hybridomas producing anti- S-100 protein MAbs were generated after immunization of Balb/c mice with purified human brain S-100 protein. The best combinations of MAbs to be used in the assay are (Fig. 17A):

Capture MAb	Detection MAb
8B10	4D2
8B10	6G1
8B10	4E3

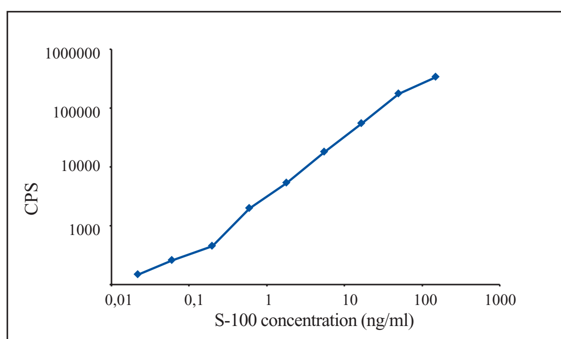


Fig. 17 A
S-100 calibration curve:
One step assay in streptavidin coated plates
Monoclonal antibodies:
- capture: *biotinylated 8B10; 200 ng/well*
- detection: *Eu-labeled 6G1; 200 ng/well*
Antigen: *S-100 protein from human brain*
Sample volume: *50 µl*
Incubation time: *20 min*
Temperature: *20°C*

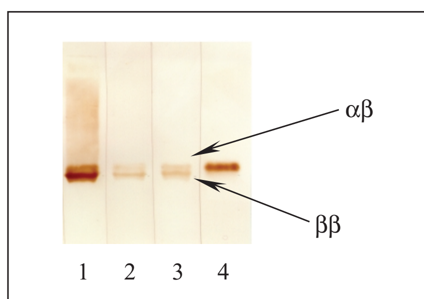


Fig. 17 B
Interaction of monoclonal antibodies with S100 protein from human brain in Western blotting (after native gel electrophoresis by Ornstein – Davis)

Gel: 20 % PAGE with 2 mM EGTA
Antigen loaded: 1µg

Lane	1	4B3
Lane	2	8B10
Lane	3	6G1
Lane	4	3B10

Performance of monoclonal antibodies with S100 protein from human brain in Western blotting is indicated on Fig. 17 B.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
S-100 Protein, Human	**4S37**	8B10	IgG1	S-100 ββ, WB, Capture, EIA
S-100 Protein, Human	**4S37**	6G1	IgG1	S-100 ββ, WB, Conjugate, EIA
S-100 Protein, Human	**4S37**	3B10	IgG2a	S-100 αβ, WB
S-100 Protein, Human **New clone**	**4S37**	4D2	IgG2a	S-100 ββ, WB, Conjugate, EIA
S-100 Protein, Human **New clone**	**4S37**	4E3	IgG2a	S-100 ββ, WB, Conjugate, EIA

HyTest

VIII Urinary Albumin

Microalbuminuria (an increased urinary albumin excretion greater or equal to 15µg/min - that is not detectable by the usual dipstick methods for macroproteinuria) predicts cardiovascular events in essential hypertensive patients, yet the pathophysiological mechanisms underlying this association remain to be elucidated.

Hypertensive patients with microalbuminuria show a higher prevalence of unfavourable left ventricular geometric patterns, depressed left ventricular function and early signs of extra-cardiac vascular damage. These findings strengthen the role of microalbuminuria as an indicator of subclinical cardiovascular disease and may account for the worse outcome that is usually associated with increased urinary albumin excretion in essential hypertension.

For detection of microalbuminuria we are offering monoclonal antibodies to human serum albumin, recombinant fragment of protein G and albumin-binding receptor.

Albumin-binding receptor protein is suitable for use as a protein for immobilization instead of anti-albumin antibodies in ELISA for detection of albumin in different human biological fluids and ligand for affinity chromatography for albumin purification.

Product	Cat. #	Purity	Source
Albumin-binding receptor	**8AB87**	>95 %	Recombinant

1. Anti-human serum albumin monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *human serum albumin*
Purification method: *protein-A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *human albumin immunoassay*

Hybridoma, producing anti-human albumin monoclonal antibody was generated after immunization of Balb/c mice with human serum albumin.

Ordering information

Product	Cat. #	Clone	Subclass	Remarks
Albumin, Human	**4T24**	1C8	IgG1	EIA

2. Recombinant protein G fragment

Source: *recombinant (E.coli)*
Purity: *≥ 95 %*
Presentation: *lyophilized*
Application: *albumin immunoassay*
Handling: *reconstitute in deionized water.*

Recombinant fragment of protein G (without antibody binding domains) makes a good match for microalbuminuria assay with anti-albumin MAb 3C8.

Ordering information

Product	Cat. #	Purity	Source
Recombinant protein G fragment	**8G1**	>95 %	Recombinant

HyTest

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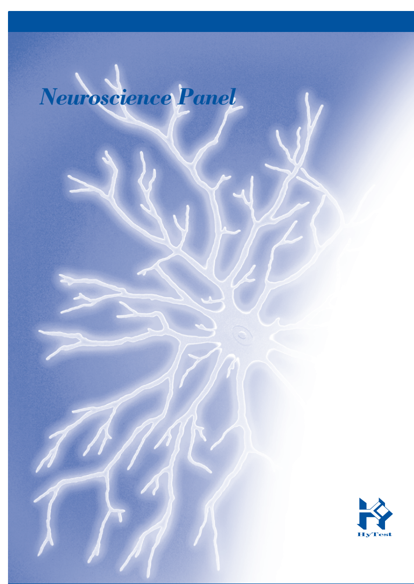

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