UPDATED JULY 7, 2004

TROPONIN I

TROPONIN T

TROPONIN C

MYOGLOBIN

FATTY ACID BINDING GLOBULIN

GLYCOGEN PHOSPHORYLASE, BB ENZYME (GPBB)

C-Reactive Protein

BRAIN S-100 Protein

ANTIBODIES, Antigens and more.....

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I Proteins of troponin complex

Troponin complex is 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 a part of troponin complex function. TnT is a tropomyosin-binding subunit which regulates the interaction of troponin complex with thin filaments; TnI inhibits ATP-ase activity of acto-myosin; TnC is a Ca^{2+} - binding subunit, playing the main role in Ca^{2+} dependent regulation of muscle contraction (1).

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 several cardiac specific isoforms of TnT (cTnT). 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 form, fast skeletal TnC isoform, is more typical for fast skeletal muscles (2).

cTnI is expressed only in myocardium. No examples of cTnI expression in healthy or injured skeletal muscle or in other tissue types are known. cTnT is probably less cardiac specific. Expression of cTnT in skeletal tissue of patients with chronic skeletal muscle injuries has been described (3).

First cTnI (4) and later cTnT (5) 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 – CK-MB (3).

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 (6).

1. Troponin I

1.1. Human cardiac troponin I (cTnI)

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

Human troponin I is presented in cardiac tissue by a single isoform with molecular weight 23876 Da and it 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 (7).

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

This 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

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 by protein kinase A) and partly phosphorylated (dephosphorylated by alkaline phosphatase) cTnI can be prepared on customer´s request.

Ordering information

Product	Cat #	Purity	Source
Human cardiac TnI	**8T53**	>95 %	Human Cardiac Muscle
Human cardiac TnI (phosphorylated)	**8T52ph**	>95 %	Human Cardiac Muscle
Human cardiac TnI (dephosphorylated)	**8T52dp**	>95 %	Human Cardiac Muscle

1.2. Troponin complex

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

Inside the cardiac troponin complex the strongest interaction between molecules has been demonstrated for cTnI – TnC binary complex especially in the presence of Ca^{2+} ($K_a=1.5\cdot10^{-8}$ M^{-1}) (8). This interaction is very important for cTnI immunodetection and should be considered by assay manufacturers. TnC, forming a complex with cTnI, changes the conformation of cTnI molecule and shields part of it´s surface, thus affecting on 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 complexes and vice versa. 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 (9).

cTnI-TnC complex formation plays an important positive role in improving the stability of cTnI molecule. cTnI, which is extremely unstable in its 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 material for standard and calibrator preparation.

In the troponin complex supplied by RDI cTnI is presented in the same form as it can be detected in the blood of AMI patients. Purification of the troponin complex is performed in mild conditions without treatment with urea containing buffers (as it usually is done for preparing 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 three main bands – cTnT, cTnI and TnC with corresponding apparent molecular weights 39, 29 and 18 kDa.

Table 1. Human cardiac troponin complex Lot 03/01 (example)

Troponin subunit	Concentration ml/ml	Molar ratio
cTnI	1.00	1
TnC	0.91	1.18
TnT	0.88	0.61

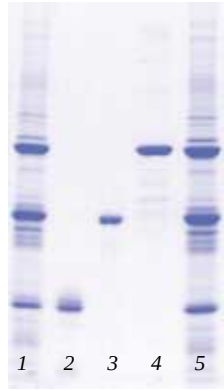


Fig. 1. SDS – gel electrophoresis of human cardiac troponin complex in reducing conditions.
From left to right:
lines 1 and 5 - cardiac troponin complex
line 2 – TnC
line 3 – cTnI
line 4 - cTnT

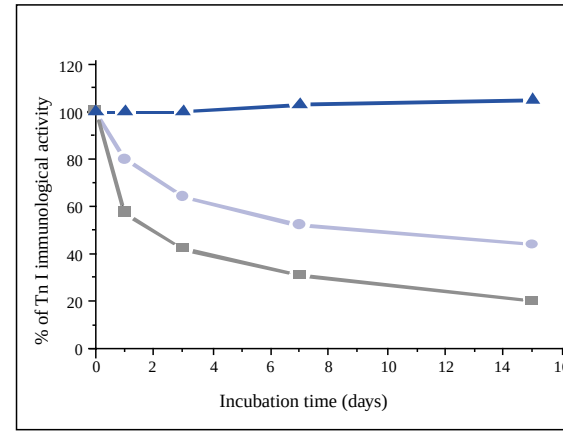


Fig. 2. Comparison of the stability of different forms of cTnI, dissolved in normal human serum and incubated for different time periods at +4°C.
- ▲ *native troponin complex*
- ● *artificial troponin complex combined from individual cTnI, cTnT and TnC*
- ■ *purified cTnI*

Advantages of native troponin complex over purified cTnI or artificial complex:

- 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

This Troponin I-T-C Complex has been chosen by AACC cTnI Standardization Subcommittee for international reference material. The standard reference material (SRM® 2921) is available from National Institute of Standards and Technology. Additional information can be found from www.nist.gov.

Ordering information

Product	Cat. #	Purity	Source
Human cardiac troponin complex	**8T62**	N/A	Human cardiac muscle

1.3. Artificial I-T-C and I-C complexes

Artificial ternary (I-T-C) and binary (I-C) complexes assembled from highly purified proteins can be prepared on customer´s request.

Ordering information

Product	Cat. #	Purity	Source
Artificial I-T-C complex	**8T62a**	N/A	Highly purified proteins
Artificial I-C complex	**8IC63a**	N/A	Highly purified proteins

1.4. Troponin I Diversity kit

Multiple cardiac troponin I (cTnI) forms can be detected in human blood. Such post-translational modifications as phosphorylation, proteolysis and complex formation with troponin C (TnC) were described in literature for cTnI molecule (10). The antibodies (mono- or polyclonal), used for the assay development should recognize different cTnI forms presented in the blood with same efficiency.

This cTnI Diversity kit includes four pairs of antigen preparations (normal/modified) to be used in the testing of assay susceptibility to different cTnI modifications - complex formation, phosphorylation, proteolysis, and presence of heparin in the sample. The kit includes free and complexed forms of cTnI, native (partially phosphorylated in vivo) and phosphorylated (in vitro) cTnI, native troponin complex and cTnI after partial proteolytic degradation and cTnI and cTnI in complex with heparin.

Ordering information

Product	Cat. #	Purity	Remarks
Troponin I Diversity kit	**K01**	N/A	Different forms of human cTnI

1.5. Troponin I Calibrator set

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

cTnI calibrators are based on isolated native troponin complex reconstituted in normal human serum. The calibrator set covers concentration range from 0 to 100 ng/ml. Each calibrator lot is checked in the stability experiments. On customer request cTnI calibrators with different antigen concentrations can be prepared.

Ordering information

Product	Cat. #	Purity	Remarks
Troponin I Calibrator Set	**8T60**	N/A	Troponin complex in normal human serum

1.6. 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. The affinity sorbent utilizes several MAbs with different epitope specificity - to eliminate from serum not only intact cTnI molecule, but also its proteolytical fragments. Based on ELISA testing cTnI level in cTnI free serum is not higher than 0.03ng/ml.

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

Ordering information

Product	Cat. #	Source
cTnI free serum	**8TFS**	Pooled normal human serum

1.7. Human skeletal troponin I (skTnI)

Source: *human skeletal tissue*
Purity: *≥ 95 %*
Composition: *slow and fast skTnI*
Presentation: *lyophilized*
Application: *negative control for cTnI specific antibodies, calibrator and standard preparation, troponin biochemical and immunochemical studies*
Remarks: *skTnI has poor solubility in buffers with physiological salt concentrations and pH values*

Antibodies utilized in human cardiac TnI assays should not have cross-reaction with skeletal isoforms of troponin I. If cross-reaction occurs wrong positive results are possible, when testing blood samples from patients with acute or chronic cardiac injuries. Taking this into consideration it is important to test the assay or antibodies used for assay development to be insensitive to the presence of skeletal TnI in the sample. This 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, fast and slow skeletal isoforms of the protein.

Skeletal isoforms of troponin I were suggested to be used as markers of acute and chronicle skeletal muscle injuries (11). In human skeletal muscles troponin I is presented by two forms – slow skeletal (186 amino acid residues, MW 21561, theoretical pI 9.61) and fast skeletal (181 amino acid residues, MW 21207, theoretical pI 8.88).

Ordering information

Product	Cat. #	Purity	Source
Human skeletal TnI	**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 antibody production, mass cTnT standard, troponin biochemical and immunochemical studies*

As well as cTnI, cardiac isoform of TnT is 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 (12).

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
Human cardiac TnT	**8T13**	>95 %	Human cardiac muscle

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 form is 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
Human skeletal TnT	**8T24**	>95 %	Human skeletal muscle

3. Troponin C

3.1. Troponin C (TnC) 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*

Two forms of troponin C (TnC) are expressed in human muscles. One is typical for slow skeletal muscles and the other for fast skeletal muscles (2). In myocardium TnC is presented by the 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 (9).

In binary cTnI-TnC complex TnC protects cTnI from protease cleavage and therefore TnC can be used as a natural stabilizer of cTnI in water solutions (13).

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
Human TnC	**8T57**	>95 %	Human cardiac muscle

4. Troponin antigens from different animal species

Troponin I and T are sensitive and specific markers of myocardial tissue damage and therefore extensively used in animal preclinical safety tests of new drugs. For assay calibration RDI offers troponin complex and isolated troponins I, T, and C purified from monkey (Macaque Cyno), canine, rat, rabbit, mouse and bovine cardiac tissue.

Ordering information

Product	Cat. #	Source
Cardiac troponin I, Monkey	**8T53mc**	Macaque Cyno cardiac muscle
Skeletal troponin I, Monkey	**8T25mc**	Macaque Cyno skeletal muscle
Cardiac troponin T, Monkey	**8T13mc**	Macaque Cyno cardiac muscle
Skeletal troponin T, Monkey	**8T24mc**	Macaque Cyno skeletal muscle
Troponin C, Monkey	**8T57mc**	Macaque Cyno cardiac muscle
Troponin complex, Monkey	**8T62mc**	Macaque Cyno cardiac muscle
Cardiac troponin I, Canine	**8T53c**	Canine cardiac muscle
Skeletal troponin I, Canine	**8T25c**	Canine skeletal muscle
Cardiac troponin T, Canine	**8T13c**	Canine cardiac muscle
Skeletal troponin T, Canine	**8T24c**	Canine skeletal muscle
Troponin C, Canine	**8T57c**	Canine cardiac muscle
Troponin complex, Canine	**8T62c**	Canine cardiac muscle
Cardiac troponin I, Rat	**8T53r**	Rat cardiac muscle
Skeletal troponin I, Rat	**8T25r**	Rat skeletal muscle
Cardiac troponin T, Rat	**8T13r**	Rat cardiac muscle
Skeletal troponin T, Rat	**8T24r**	Rat skeletal muscle
Troponin C, Rat	**8T57r**	Rat cardiac muscle
Troponin complex, Rat	**8T62r**	Rat cardiac muscle
Cardiac troponin I, Bovine	**8T53b**	Bovine cardiac muscle
Skeletal troponin I, Bovine	**8T25b**	Bovine skeletal muscle
Cardiac troponin T, Bovine	**8T13b**	Bovine cardiac muscle
Skeletal troponin T, Bovine	**8T24b**	Bovine skeletal muscle
Troponin C, Bovine	**8T57b**	Bovine cardiac muscle
Troponin complex, Bovine	**8T62b**	Bovine cardiac muscle
Cardiac troponin I, Mouse	**8T53m**	Mouse cardiac muscle
Skeletal troponin I, Mouse	**8T25m**	Mouse skeletal muscle
Cardiac troponin T, Mouse	**8T13m**	Mouse cardiac muscle
Skeletal troponin T, Mouse	**8T24m**	Mouse skeletal muscle
Troponin C, Mouse	**8T57m**	Mouse cardiac muscle
Troponin complex, Mouse	**8T62m**	Mouse cardiac muscle
Cardiac troponin I, Rabbit	**8T53rb**	Rabbit cardiac muscle
Skeletal troponin I, Rabbit	**8T25rb**	Rabbit skeletal muscle
Cardiac troponin T, Rabbit	**8T13rb**	Rabbit cardiac muscle
Skeletal troponin T, Rabbit	**8T24rb**	Rabbit skeletal muscle
Troponin C, Rabbit	**8T57rb**	Rabbit cardiac muscle
Troponin complex, Rabbit	**8T62rb**	Rabbit cardiac muscle

Information about antibodies for the detection of troponin antigens from different animal species can be found from the part: II TROPONIN SPECIFIC ANTIBODIES, 1.1.2 Immunoassays for the detection of cTnI from different animal species.

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*
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 free cTnI, cTnI peptides or with cTnI in the form of native troponin complex. MAbs were tested not to have cross-reaction with skeletal forms of the protein as well as with cTnT and TnC. The ability of the antibodies to recognize complexed and free forms of the protein has been checked. Some antibodies are specific to both (complexed and free forms) of cTnI and some are affected by complex formation in different degree. MAb 414 recognizes only free form of the molecule. MAbs 6F9 and C5 recognize both cardiac and skeletal forms of human TnI.

The epitope specificity of all MAbs was precisely determined by SPOT technique or by other methods utilizing different peptide libraries (Fig. 3). We have currently available antibodies specific to almost all parts of cTnI molecule and these antibodies could be used as well for scientific studies as for the development of new generations of cTnI assays.

In Table 2 you can find data demonstrating the cross-reactivity of MAbs with cTnI from several animal species. Majority of the presented monoclonal antibodies can be used for immunodetection of cTnI from different animal species.

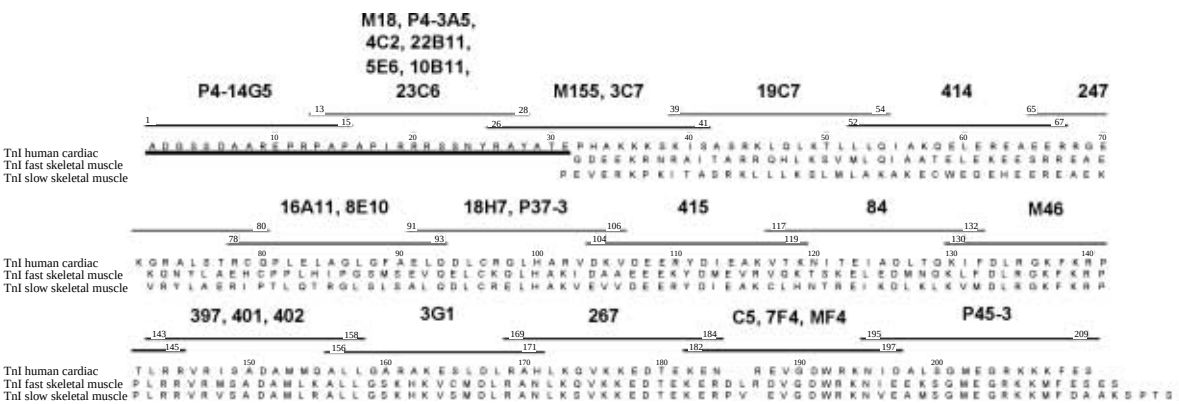


Fig. 3 Epitope mapping of anti-cTnI monoclonal antibodies

Clone	Cross-reaction (in Western blotting)									
	Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
10B11	+	-	+	-	+	+	+	+	-	-
22B11	++	-	+	-	-	-	-	-	-	-
4C2	++	++	++	++	++	++	+	++	++	-
19C7	++	++	+	++	+	++	++	++	+	++
414	+	+	+	-	+	+	-	-	+	+
8E10	+	+	+	+	+	+	+	-	-	-
16A11	+	+	+	+	+	+	+	-	-	-
18H7	+	+	+	+	+	+	+	+	+	-
MF4	+	+	+	+	+	-	+	+	+	-
247	++	++	++	++	++	+	++	++	++	N/A
P45-3	++	++	++	++	++	++	++	++	++	++

Table 2. Cross-reaction of anti-cTnI Mabs with antigens from different animal species

Ordering information

Clone	Cat. #	Specificity	Subclass	Epitope	Application
P4-14G5	4T21	cTnI	IgG1	1-15 a.a.r.	EIA, Sandwich immunoassay, WB
P4-3A5	4T21	cTnI	IgG1	13-29 a.a.r.	EIA, Sandwich immunoassay, WB
M18	4T21	cTnI	IgG1	13-29 a.a.r.	EIA, Sandwich immunoassay (capture), WB
23C6	4T21	cTnI	IgG2a	15-25 a.a.r.	EIA, Sandwich immunoassay, WB
10B11	4T21	cTnI	IgG1	16-20 a.a.r.	EIA, Sandwich immunoassay, WB
4C2	4T21	cTnI	IgG2a	23-29 a.a.r.	EIA, Sandwich immunoassay (capture, detection), WB
3C7	4T21	cTnI	IgG1	25-40 a.a.r.	EIA, Sandwich immunoassay (detection), WB
M155	4T21	cTnI	IgG1	27-43 a.a.r.	EIA, Sandwich immunoassay (detection), WB
19C7	4T21	cTnI	IgG2b	41-49 a.a.r.	EIA, Sandwich immunoassay (capture, detection), WB
414	4T21	cTnI	IgG1	56-61 a.a.r.	EIA, Sandwich immunoassay, WB
247	4T21	cTnI	IgG1	65-80 a.a.r.	EIA, Sandwich immunoassay
8E10	4T21	cTnI	IgG1	87-91 a.a.r.	EIA, Sandwich immunoassay (capture, detection), WB
16A11	4T21	cTnI	IgG1	87-91 a.a.r.	EIA, Sandwich immunoassay (capture, detection), WB
P37-3	4T21	cTnI	IgG	91-106 a.a.r.	EIA, Sandwich immunoassay
18H7	4T21	cTnI	IgG1	102-108 a.a.r.	EIA, Sandwich immunoassay (detection), WB
415	4T21	cTnI	IgG	104-119 a.a.r.	EIA, Sandwich immunoassay
84	4T21	cTnI	IgG	117-132 a.a.r.	EIA, Sandwich immunoassay
M46	4T21	cTnI	IgG1	130-145 a.a.r.	EIA, Sandwich immunoassay, WB
397	4T21	cTnI	IgG	143-158 a.a.r.	EIA, Sandwich immunoassay
401	4T21	cTnI	IgG	143-158 a.a.r.	EIA, Sandwich immunoassay
402	4T21	cTnI	IgG	143-158 a.a.r.	EIA, Sandwich immunoassay
3G1	4T21	cTnI	IgG2b	156-171 a.a.r.	EIA, Sandwich immunoassay
267	4T21	cTnI	IgG	169-184 a.a.r.	EIA, Sandwich immunoassay
C5	4T21	cTnI	IgG2b	186-192 a.a.r.	EIA, Sandwich immunoassay
7F4	4T21	cTnI	IgG1	190-196 a.a.r.	EIA, Sandwich immunoassay, WB
MF4	4T21	cTnI	IgG1	190-196 a.a.r.	EIA, Sandwich immunoassay (capture, detection), WB
P45-3	4T21	cTnI	IgG2b	195-209 a.a.r.	EIA, Sandwich immunoassay
5E6	4T45	Phosphory-lated cTnI	IgG2b	N/A	EIA, Sandwich immunoassay, WB
22B11	4T46	Dephospho-rylated cTnI	IgG2b	20 – 24 a.a.r.	EIA, Sandwich immunoassay, WB

New high affinity antibody clones will be available in September 2004. Please contact our customer service for additional information.

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 are:

19C7	——	16A11
19C7 + MF4 (mixture for coating)	——	16A11 + 7B9 (TnC specific)
19C7 + 4C2 (mixture for coating)	——	16A11 + 7B9 (TnC specific)
19C7 + M18 (mixture for coating)	——	MF4 + 8E10 (mixture for detection)
19C7 + M18 (mixture for coating)	——	MF4 + 16A11 (mixture for detection)

The pairs demonstrate high sensitivity (0.02 ng/ml, Fig. 4), good kinetics, low background and high reproducibility. Using combinations of MAbs (2 – 3 antibodies with different epitope specificity) as capture and 2 – 3 MAbs as detection the sensitivity of sandwich immunoassays can be increased 2 – 4 fold. Immunoassays, based on these recommended antibodies, recognize complexed and free forms of the antigen with same sensitivity. Recently it was demonstrated that during 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. For better sensitivity and reproducibility for assay designing we recommend to use antibodies that recognize the stable part of the molecule or combination of antibodies (>1 MAbs as capture and detection, specific to different parts of the molecule. Even when you are using a combination of MAbs for capture and detection it is desirable to have among these antibodies one which is specific to the stable part of the molecule.

Since the main part (> 95 %) of cTnI in human blood is presented as a binary cTnI-TnC complex, the antibodies utilized in the assay should recognise cTnI in complex with TnC. Because the amount of complexed cTnI in blood of AMI patients is very high, for cTnI immunodetection we recommend using also a MAb that is specific to TnC or a MAb specific to TnC together with MAb(s) specific to cTnI. Such approach helps improving the sensitivity and reproducibility of cTnI immunoassays. For additional information about other MAb combinations tested in cTnI immunoassay please contact RDI customer service department.

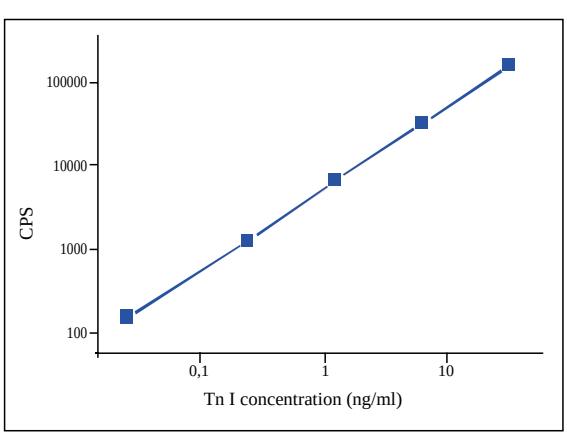
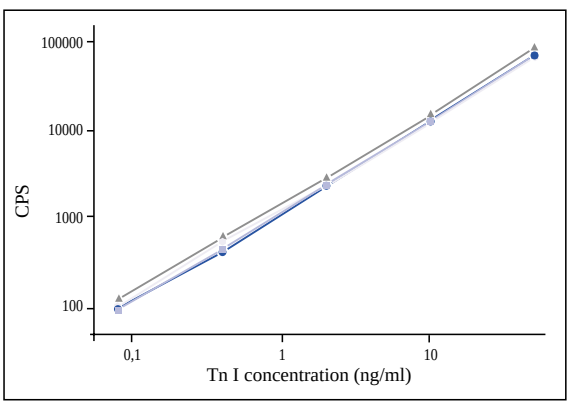


Fig. 4. cTnI calibration curve:
One step assay in streptavidin coated plates
Monoclonal antibodies:
- capture: biotinylated 19C7; 200 ng/well
- detection: Eu-labeled 8E10; 200 ng/well
Antigen: cTnI (native troponin complex)
Incubation time: 20 min
Temperature: 20°C

In assay designing it should be taken into consideration that the assay should not be affected by partial proteolytic degradation of cTnI molecule, oxidation-reduction, phosphorylation, complex formation with TnC, presence of heparin in the samples and negative interference (15). The antibody combinations that we are recommending are not affected by any of these factors. The antibodies are validated for traditional types of sandwich immunoassays as well as for immunochromatography methods.

1.1.2. Immunoassays for the detection of cTnI from different animal species

Clinical studies of new drugs and new methods of surgery are carried out on experimental animals. The effects of the new technologies on cardiac function and on cardiac myocyte viability can be important. As it is seen in Table 2, the majority of anti-cTnI MAbs has wide specificity and recognizes not only human cTnI but also cardiac TnI from other species. Several combinations of MAbs were tested and could be used for quantitative detection of cTnI in blood of different species. In Fig. 5 we are presenting the calibration curve for the assay utilizing MAb M155 as a capture and MAb 19C7 as detection. E.g. that MAb combination gives equal response with antigens from different animal species. Other MAb pairs, with epitopes located in different parts of troponin molecule, could also be used for the same purpose.



- *human cTnI (in native complex)*
- *rat cTnl (in native complex)*
- *canine cTnI (in native complex)*
- *mouse cTnI (in native complex)*

Fig. 5. cTnI calibration curve:
One step assay in streptavidin coated plates
Monoclonal antibodies:
- capture: biotinylated M155; 200 ng/well
- detection: Eu-labeled 19C7; 200 ng/well

Antigens:	*Human, Rat, Canine, Mouse (native troponin complex)*
Incubation time:	*20 min*
Temperature:	*20°C*

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

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

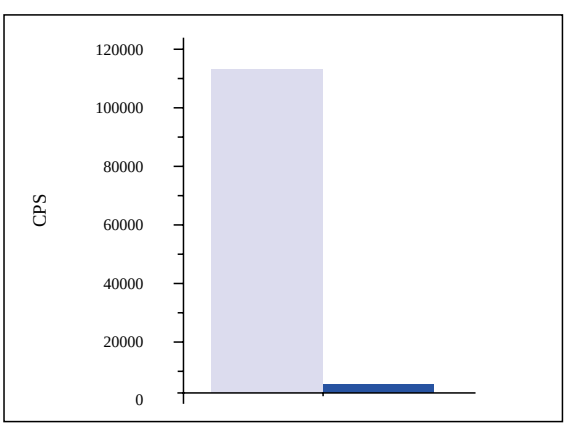
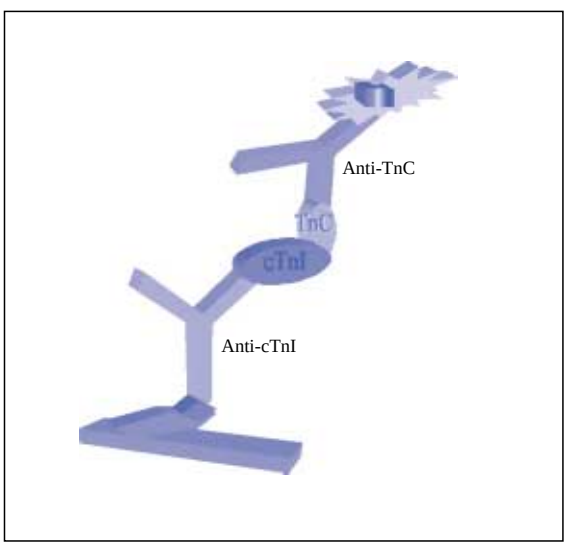


Fig. 6. Detection of free cTnI
Two forms of cTnI detected by sandwich immunoassay utilizing MAb 10F4 as capture and MAb 414 as detection.
Blue column - free cTnI (30 ng/ml)
Dark blue column - cTnI (30 ng/ml) in binary cTnI - TnC complex

1.1.4. Detection of binary cTnI-TnC complex

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

A.



B.

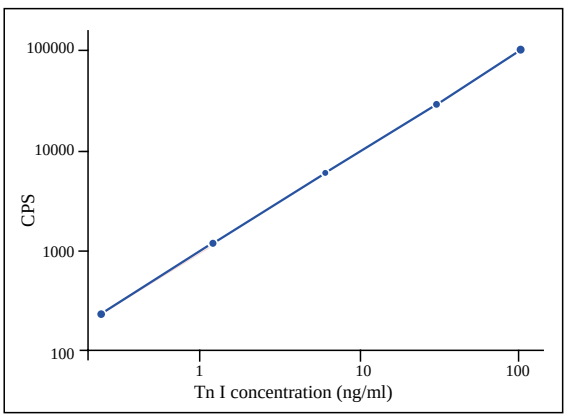
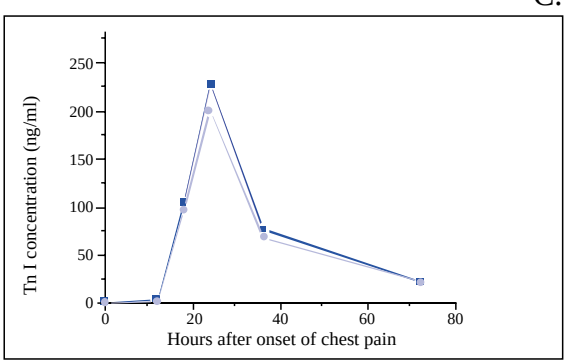


Fig. 7. Measurement of cTnI-TnC complex by mixed assay
A. Scheme of the assay
B. Calibration curve (ternary native complex as a standard)
C. cTnI concentration in the form of cTnI-TnC complex (circles; measured by mixed assay) and total cTnI concentration (free and complexed - square) measured in AMI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

C.



1.1.5. Detection of binary cTnI-cTnT complex

Similar approach that was recommended for quantitative detection of cTnI-TnC complex can be used also for cTnI-cTnT complex detection. We recommend using anti-cTnT MAb 1F2 as capture and anti-cTnI MAb 19C7 as detection. Such assay has no cross-reaction with free forms of cTnI, cTnT and TnC, and recognizes only cTnI-cTnT binary and cTnI–cTnT–TnC ternary complexes.

1.1.6. Detection of dephosphorylated cTnI

cTnI is phosphorylated in vivo and it can also be phosphorylated in vitro by cAMP-dependent protein kinase A. Sites of phosphorylation are the serines in the 22nd and 23rd position (14). Some RDI MAbs recognize the epitopes containing these two amino acid residues. It was demonstrated recently that MAb 22B11 recognizes only dephospho- form of cTnI and does not react with mono- or biphosphorylated form of the antigen (Fig. 8). 22B11 can be used for qualitative or semi-quantitative immunodetection of dephosphorylated cTnI in Western blotting and 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).

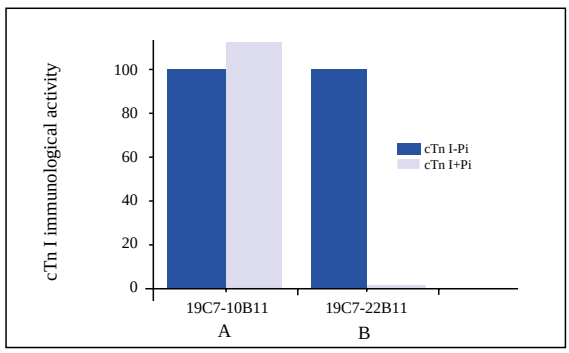


Fig. 9. Two immunoassays recognizing dephospho-(dark blue columns) and biphospho-(light blue columns) forms of cTnI.

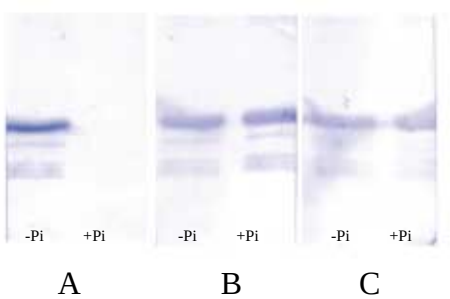


Fig. 8. Monoclonal antibodies 22B11(A), 10B11(B) and 8E10 (C), recognizing dephospho- and phospho- forms of human cardiac TnI.

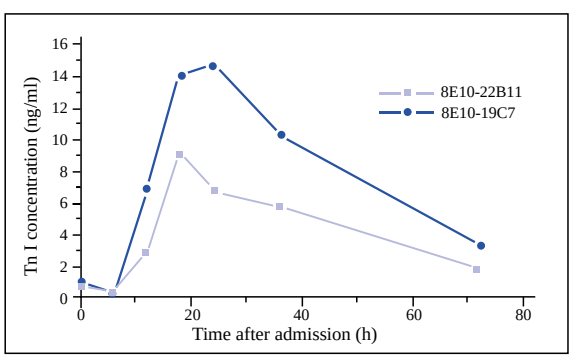


Fig.10. cTnI measurements: total (circles) and dephosphorylated (squares) cTnI in serial blood samples from AMI patient.

1.1.7. Detection of cTnI or cTnI fragments by Western Blotting

All anti-cTnI MAbs recognize human cTnI or cTnI fragments in Western blotting (Fig. 11). For better sensitivity in Western blotting we recommend using MAbs 4G6, 19C7, 16A11 and MF4.

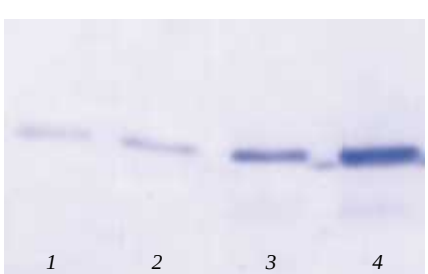


Fig. 11. Detection of cTnI by Western blotting
After SDS-PAGE cTnI was transferred on nitrocellulose membrane and probed by 16A11 MAbs and then by hrp-conjugated goat anti-mouse IgG antibodies. Substrate - diaminobenzidine.
Lane 1 - 3 ng cTnI
Lane 2 - 10 ng cTnI
Lane 3 - 30 ng cTnI
Lane 4 - 100 ng cTnI

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*

Polyclonal antibodies to cTnI were generated after immunization with purified cTnI protein and with cTnI in the form of native troponin complex. Polyclonal antibodies are purified by immunoaffinity method utilizing cTnI-agarose as an affinity matrix.

Polyclonal antibodies, generated after immunization by different cTnI peptides are also available on customer request. For more information about polyclonal antibodies contact our customer service department.

Ordering information

Product	Cat. #
Polyclonal anti-cTnI antibody	**4T21/2**

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 cTnT. MAbs were tested to have no cross-reaction with cTnI and TnC.

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

Cross-reaction of anti-human cTnT MAbs with cTnTs from other species was studied in Western blotting with crude extracts of cardiac tissue from different species (Table 3).

Clone	Cross-reaction (in Western blotting)									
	Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
1F2	+	+	-	+	+	+	+	-	-	+
7F4	++	N/A	++	N/A	-	-	-	N/A	N/A	-
7G7	+	+	-	-	-	-	-	-	-	-
3D6	+	+	-	+	+	+	-	-	+	+
2F3	++	+	++	++	+	+	+	+	+	+
2G3	++	+	+	+	+	+	+	+	+	-
6G9	+	+	-	+	+	+	-	+	+	+
1F11	++	++	++	++	+	+	+	+	+	+
1A11	++	++	++	++	+	+	+	+	++	+
7A9	+	+	-	+	+	+	-	-	-	-

Table 3. Anti-cTnT monoclonal antibodies

Ordering information

Clone	Cat. #	Specificity	Subclass	Epitope	Application
9G6	4T19	cTnT	IgG1	1 – 60 a.a.r.	EIA, Sandwich immunoassay, WB
1F2	4T19	cTnT	IgG1	60 – 70 a.a.r.	EIA, Sandwich immunoassay, WB
7G7	4T19	cTnT	IgG1	60 – 70 a.a.r.	EIA, Sandwich immunoassay, WB
7F4	4T19	cTnT	IgG2b	61 – 70 a.a.r.	EIA, Sandwich immunoassay, WB
3D6	4T19	cTnT	IgG1	70 – 94 a.a.r.	EIA, Sandwich immunoassay, WB
2G3	4T19	cTnT	IgG2b	94 – 180 a.a.r.	EIA, Sandwich immunoassay, WB
6G9	4T19	cTnT	IgG1	94 – 180 a.a.r.	EIA, Sandwich immunoassay, WB
1C11	4T19	cTnT	IgG1	95 – 181 a.a.r.	EIA, Sandwich immunoassay, WB
1F11	4T19	cTnT	IgG2b	146 – 160 a.a.r.	EIA, Sandwich immunoassay, WB
1A11	4T19	cTnT	IgG2b	146 – 160 a.a.r.	EIA, Sandwich immunoassay, WB
7A9	4T19	cTnT	IgG1	180 – 287 a.a.r.	EIA, Sandwich immunoassay, WB
5C12	4T19	cTnT	IgM	180 – 258 a.a.r.	EIA, Sandwich immunoassay, WB
2F3	4T19	cTnT	IgG2b	N/A	EIA, Sandwich immunoassay, WB
7E7	4T19	cTnT	IgG	N/A	EIA, Sandwich immunoassay, WB

4. Detection of cTnT from different animal species

cTnT as a sensitive and reliable marker of myocardial cell death is widely used in preclinical studies of new drugs, experimental cardiology and other studies performed on experimental animals to demonstrate the effect of artificial intervention on the viability of myocardial cells.

RDI offers a combination of two monoclonal antibodies suitable for the development of sandwich immunoassay for quantitative detection of human, canine, rat and mouse cTnTs in blood samples (Fig. 12). The antibodies were chosen on the basis of their reproducibility, reliability and reactivity with cTnTs from different animal species. The epitope of MAb 1C11 is located in the 95-181 region of cTnT sequence. This part of cTnT molecule is very conservative and has a high degree of similarity between human, rat and mouse TnTs. Therefore MAb 1C11 reacts equally well with these antigens.

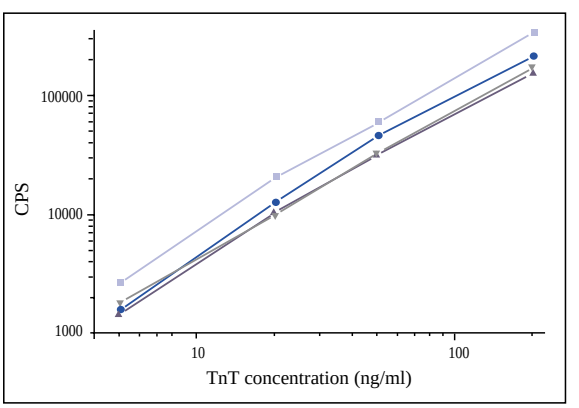


Fig. 12. Calibration curves for sandwich cTnT fluoroimmunoassay with different animal TnTs as antigen:

(-●-) canine
(-■-) human
(-▼-) mouse
(-▲-) rat

Monoclonal antibodies:
capture: 1C11, 1 µg/well
detection: 7E7, 200 ng/well
Assay time: 30 min
Room temperature
Hybridoma cell line producing MAb 7E7 was raised after hybridization of myeloma cell line sp2/0 with splenocytes of mice immunized with human tertiary Tn complex. Thus MAb 7E7 demonstrates slight cross-reaction with cTnI (about 5 % based on ELISA data).

5. Anti-TnC 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 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

Clone	Cat. #	Specificity	Subclass	Application
1A2	4T27	TnC	IgG2a	EIA, Sandwich immunoassay (capture), WB
7B9	4T27	TnC	IgG1	EIA, Sandwich immunoassay(detection), WB
12G3	4T27	TnC	IgG2b	EIA, Sandwich immunoassay, WB

References:
1. *Gomes, A.V, Potter,J.D., Szczesna-Cordary, D. (2002) The role of Troponin in muscle contraction. Life, 54: 323-333.*
2. *Marston,S.B., Redwood,C.S. (2003) Modulation of thin filament activation by breakdown or isoform switching of thin filament Proteins. Circ. Res., 93: .1170-1178*
3. *Sarko J, Pollack CV Jr. (2002) Cardiac troponins. J Emerg Med. 2002, 23(1), pp. 57-65*
4. *Cummins B, Auckland ML, Cummins P. (1987) Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J. 113(6): 1333-1344*
5. *Katus HA, Remppis A, Looser S, Hallermeier K, Scheffold T, Kubler W. (1989) Enzyme linked immunoassay of cardiac troponin T for the detection of acute myocardial infarction in patients. J Mol Cell Cardiol. 21(12): 1349-1353*
6. *Hamm CW. (2001) Acute coronary syndromes. The diagnostic role of troponins. Thromb Res., 103 Suppl 1:S63-9*
7. *Labugger R, Organ L, Collier C, Atar D, Van Eyk JE. (2000) Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. Circulation. 102(11):1221-1226*
8. *Reiffert SU, Jaquet K, Heilmeyer LM Jr, Herberg FW (1998) Stepwise subunit interaction changes by mono- and bisphosphorylation of cardiac troponin I. Biochemistry, 37(39): 13516-13525.*
9. *Katrukha AG, Bereznikova AV, Esakova TV, Pettersson K, Lovgren T, Severina ME, Pulkki K, Vuopio-Pulkki LM, Gusev NB. (1997) Troponin I is released in bloodstream of patients with acute myocardial infarction not in free form but as complex. Clin. Chem. 43(8): 1379-1385.*
10. *Wu AH, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, Bodor G. (1998) Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. Clin. Chem. , 44(6): 1198-1208.*
11. *Onuoha GN, Alpar EK, Dean B, Tidman J, Rama D, Laprade M, Pau B.(2001) Skeletal troponin-I release in orthopedic and soft tissue injuries. J Orthop Sci. 6(1):11-15.*
12. *Bertinchant JP, Robert E, Polge A, Marty-Double C, Fabbro-Peray P, Poirey S, Aya G, Juan JM, Ledermann B, de la Coussaye JE, Dauzat M. (2000) Comparison of the diagnostic value of cardiac troponin I and T determinations for detecting early myocardial damage and the relationship with histological findings after isoprenaline-induced cardiac injury in rats. Clin Chim Acta. 298(1-2):13-28.*
13. *Katrukha AG, Bereznikova AV, Filatov VL, Esakova TV, Kolosova OV, Pettersson K, Lovgren T, Bulargina TV, Trifonov IR, Gratsiansky NA, Pulkki K, Voipio-Pulkki LM, Gusev NB.(1998) Degradation of cardiac troponin I: implication for reliable immunodetection. Clin. Chem. 44(12): 2433-2440.*
14. *Jaquet K, Thieleczek R, Heilmeyer LM Jr. (1995) Pattern formation on cardiac troponin I by consecutive phosphorylation and dephosphorylation. Eur J Biochem. 231(2):486-90.*
15. *Katrukha AG. (2003) Antibody selection strategies in cardiac troponin assays. Cardiac Markers, 2nd edition, Edited by Alan HB. Wu. 173-185.*

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*

Myoglobin is a small heme-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 tissues. Only one form of myoglobin is expressed in cardiac and skeletal muscles.

Myoglobin is known as a marker of myocardial damage and it has been used for more than three decades (1). Nowadays it still is very commonly used in clinical practice as an early marker of AMI (2). It appears in patients' blood 1 – 3 hours after onset of the symptoms, reaching peak level within 8 – 12 hours (3). 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 concentration in blood (4). Thus myoglobin is used together with cTnI or cTnT in clinical practise for better specificity in AMI diagnosis.

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 (Fig. 13).

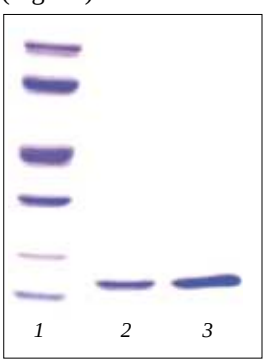


Fig. 13. SDS-PAGE of human myoglobin in reducing conditions.
Lane 1 - Molecular weight standards, Pharmacia (94, 67, 43, 30, 20 and 14.4)
Lanes 2 and 3 - Human myoglobin 3µg and 6µg, respectively
Gel staining: Coomassie brilliant blue R-250

In the year 2001, This myoglobin preparation was selected by International Federation of Clinical Chemistry as an International Standard Material.

Ordering information

Product	Cat. #	Purity	Source
Human 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 can be used for the immunoassay development. The best MAb combinations for sandwich immunoassay are:

4E2 ——— 7C3
7C3 ——— 4E2
4E2 ——— 908

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
4E2	4M23	Myoglobin	IgG1	EIA, Sandwich immunoassay (capture)
7C3	4M23	Myoglobin	IgG1	EIA, Sandwich immunoassay (detection)
908	4M23	Myoglobin	IgG1	EIA, Sandwich immunoassay (detection)

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. Based on ELISA testing the level of myoglobin in myoglobin free serum is below 0.1 ng/ml (Fig. 14).

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

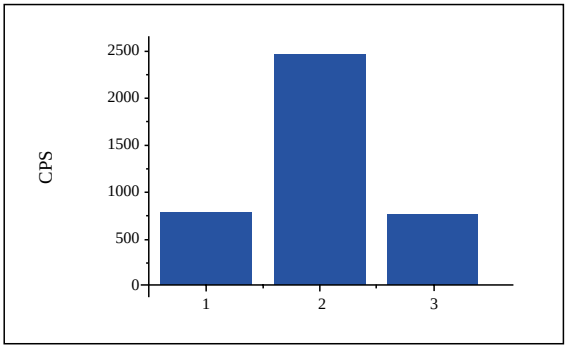


Fig. 14. Myoglobin level in normal human serum and in myoglobin free serum detected in sandwich-immunoassay.
1 - Buffer
2 - Pooled normal human serum before myoglobin extraction (4 ng/ml of myoglobin)
3 - Residual signal in myoglobin free serum

Ordering information

Product	Cat. #	Source
Myoglobin free serum	**8MFS**	Pooled normal human serum

References:
1. *Kagen L, Scheidt S, Butt A. (1977) Serum myoglobin in myocardial infarction: the "staccato phenomenon." Is acute myocardial infarction in man an intermittent event? Am J Med. 62(1):86-92.*
2. *Penttila K. et al. (2002) Myoglobin, creatine kinase MB isoforms and creatine kinase MB mass in early diagnosis of myocardial infarction in patients with acute chest pain. Clin Biochem. 35(8):647-53.*
3. *Srinivas VS. et al. (2001) Myoglobin levels at 12 hours identify patients at low risk for 30-day mortality after thrombolysis in acute myocardial infarction: a Thrombolysis in Myocardial Infarction 10B substudy. Am Heart J. 142(1):29-36.*
4. *Van Nieuwenhoven FA. Et al. (1995) Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. Circulation. 92(10):2848-54*

IV Pregnancy Associated Plasma Protein A (PAPP-A)

1. Human PAPP-A

Source: *human retroplacental blood*
Purity: *> 90 % by SDS-PAGE*
Presentation: *lyophilized*
Composition: *ternary complex (2+2) pregnancy-associated plasma protein A (PAPP-A) in complex with proform of major basic protein (proMBP)*
Application: *PAPP-A calibrators and standards, PAPP-A immunochemical and biochemical studies, immunogen for antibody production*

PAPP-A was originally isolated from pregnancy serum as heterotetrameric protein complex with molecular weight approximately 500 kDa, consisting of two PAPP-A subunits disulfide-linked with two subunits of the proform of eosinophil major basic protein (proMBP). In such a complex proMBP functions as an inhibitor of PAPP-A proteinase activity. PAPP-A is widely used as a marker of some pathologies during pregnancy. Reduced level of PAPP-A in pregnancy blood in the first trimester is associated with fetal Down syndrome. PAPP-A is considered as one of the best biochemical markers in early pregnancy used to screen for Down syndrome during the first trimester (1).

Recently a dimeric form (homodimer) of pregnancy-associated plasma protein A (dPAPP-A) was described. It consists of two 1547-residue, disulfide-linked, glycosylated subunits and functions as zinc-binding metalloproteinase (2).

Recent data show that dPAPP-A might be utilized as a marker of acute coronary syndrome. dPAPP-A is abundantly expressed in atherosclerotic plaque cells and can be found in the extracellular matrix of ruptured and eroded unstable plaques but not in stable plaques (3). dPAPP-A is responsible for cleavage of insulin-like growth factor binding protein (IGFBP-4), thus causing release of bound IGF (2). In that way dPAPP-A constitutes a specific activator of insulin-like growth factor, a mediator of atherosclerosis. Serum levels of dPAPP-A are also elevated in patients with myocardial infarction and unstable angina, but not with stable angina. Therefore serum dPAPP-A levels can identify patients in the beginning of the process of plaque instability (4).

This PAPP-A is purified from retroplacental blood and is presented as a heterotetrameric complex with proMBP (Fig. 15).

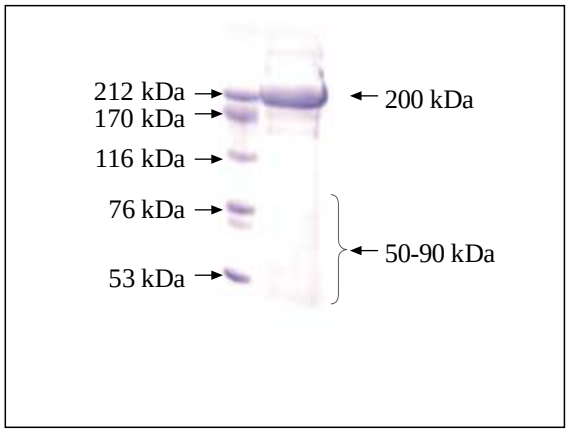


Fig. 15. SDS-gel electrophoresis of PAPP-A in reducing conditions.
Lane 1 - molecular weight standards,
Lane 2 - human PAPP-A (3 µg)
Gel staining: Coomassie brilliant blue R-250

Comments: proMBP subunit migrates as a diffuse spot with molecular mass around 50 – 90 kDa and is hardly stained by Coomassie brilliant blue because of high degree of glycosylation (~ 40%)

Ordering information

Product	Cat. #	Purity	Source
Human PAPP-A (heterotetramer)	**8P64**	>90 %	Human retroplacental blood

2. Anti-PAPP-A monoclonal antibodies

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

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of Balb/c mice immunized with purified human PAPP-A (heterotetramer) antigen purified from retro-placental blood.

Specificity of antibodies was confirmed by ELISA and Western blotting. All antibodies recognize antigen in ELISA. MAb 18A10 recognizes PAPP-A in Western Blotting after SDS-gel electrophoresis in reducing conditions. MAbs 4G11, 10A5, 18A10 and 10E1 recognize heterotetrameric complex in Western Blotting after SDS-electrophoresis in non-reducing conditions (Fig 16). MAb 11E4 is specific to the proMBP component of the heterotetramer and recognizes proMBP in Western Blotting after SDS-gel electro-phoresis in reducing conditions.

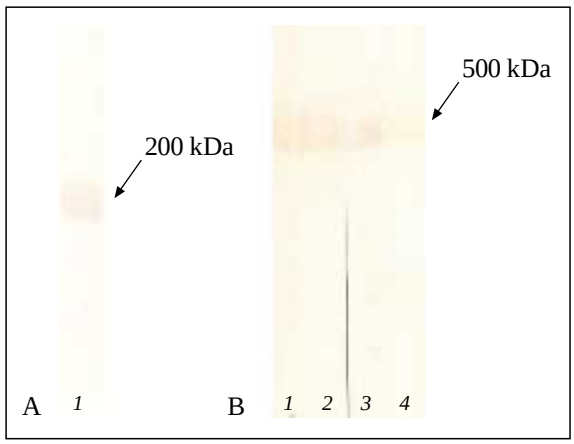


Fig. 16. Detection of human PAPP-A by different monoclonal antibodies in Western Blotting.
A. 1 - MAb 18A10 recognizes PAPP-A subunit after SDS-gel electrophoresis in reducing conditions (gradient gel 5 % – 15 %)
B. 1 - 4 MAbs 4G11, 10A5, 18A10 and 10E1 recognize heterotetrameric complex (gradient gel 2.5 % – 10 %)

Recommended pairs for the detection of PAPP-A in blood of AMI patients and patients with unstable angina (capture/detection):

4G11 —— 3C8 (Fig. 17)
4G11 —— 10H9 (Fig. 17)
10E2 —— 10E1

Best pairs for the detection of PAPP-A in pregnancy serum:

4G11 —— 3C8
4G11 —— 10H9
10A5 —— 3C8
10E2 —— 10E1
10E2 —— 5H9
5H9 —— 10E2

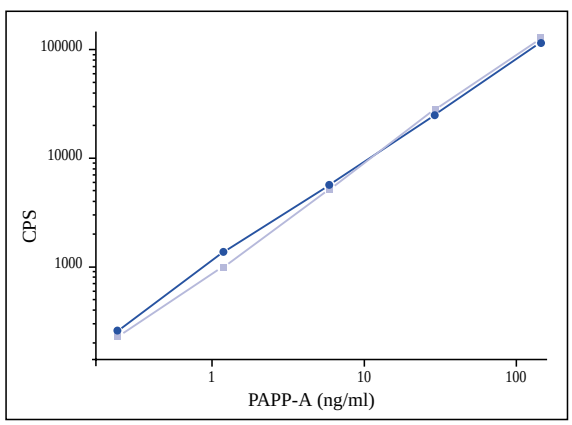


Fig. 17. PAPP-A calibration curve for two assays
Capture: *biotinylated 4G11; 200 ng/well*
Detection: *Eu-labeled 3C8 (-■-); 400 ng/well; Eu-labeled 10H9 (-●-), 200 ng/well*
Streptavidin coated plate
Assay time: *30 min*
Room temperature

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
10E1	4P41	PAPP-A	IgG2b	EIA, Sandwich immunoassay (detection), WB
10E2	4P41	PAPP-A	IgG2b	EIA, Sandwich immunoassay (capture, detection)
5H9	4P41	PAPP-A	IgG2b	EIA, Sandwich immunoassay (capture, detection)
4G11	4P41	PAPP-A	IgG2a	EIA, Sandwich immunoassay (capture), WB
10A5	4P41	PAPP-A	IgG2a	EIA, Sandwich immunoassay (capture), WB
3C8	4P41	PAPP-A	IgG2a	EIA, Sandwich immunoassay (detection)
10H9	4P41	PAPP-A	IgG2a	EIA, Sandwich immunoassay (detection)
18A10	4P41	PAPP-A	IgG2a	EIA, Sandwich immunoassay, WB
11E4	4P41	proMBP	IgG	EIA, Sandwich immunoassay, WB

References:
1. *Wald, N. J. et al. First trimester biochemical screening for Down's syndrome.// Ann Med, 26 (1), 23-9 (1994).*
2. *Lawrence, J. B et al The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A.// Proc Natl Acad Sci U S A, 96 (6), 3149-53 (1999).*
3. *Bayes-Genis, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes.// N Engl J Med, 345 (14), 1022-9 (2001).*
4. *Qin, Q. P., Laitinen, P., Majamaa-Voltti, K., Eriksson, S., Kumpula, E. K., and Pettersson, K. Release Patterns of Pregnancy Associated Plasma Protein A (PAPP-A) in Patients with Acute Coronary Syndromes.// Scand Cardiovasc J, 36 (6), 358-61 (2002).*

V Human Brain Natriuretic Peptide (BNP)

BNP is circulating in blood as a peptide hormone with natriuretic, vasodilatory and renin inhibitory properties. BNP is secreted predominantly by the left ventricular myocytes in response to volume expansion and pressure overload. BNP belongs to a family of structurally similar peptide hormones, which includes atrial natriuretic peptide (ANP), BNP, C-type natriuretic peptide (CNP) and urodilatin. These peptides are characterized by a common 17 amino acid ring structure with a disulfide bond between two cystein residues. This ring structure shows high homology between different natriuretic peptides (eleven of the 17 amino acid residues are homologous in the ring of each of the natriuretic peptides, see fig. 18). BNP is a 32 amino acid peptide with disulfide bond between the cystein residues Cys10 and Cys26. In earlier studies it has been demonstrated that BNP concentration in blood increases with the severity of congestive heart failure. Quantitative measurement of BNP in blood provides an objective indicator of congestive heart failure severity (1, 2).

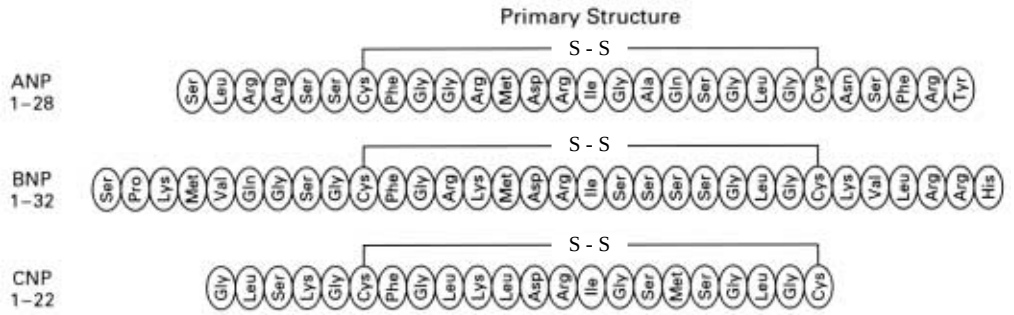


Fig. 18. Structures of human ANP, BNP and CNP.

1. Anti-BNP monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp 2/0*
Antigen: *synthetic human BNP 1-32*
Specificity: *human BNP and proBNP*
Purification method: *protein A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *BNP and proBNP immunoassay, BNP and proBNP immunodetection in Western blotting*

Hybridoma cell lines producing MAbs were derived from hybridization of Sp2/0 myeloma cells with spleen cells of BALB/c mice immunized with human synthetic BNP 1-32, conjugated to carrier protein. Specificity of antibodies was confirmed by ELISA. All antibodies recognize antigen in ELISA. MAb 1B6 recognizes proBNP in Western Blotting after tricine-SDS-gel electrophoresis in reducing conditions.

All MAbs can be used in sandwich immunoassay as detection antibodies in combination with polyclonal rabbit antibodies, specific to amino acids 1-10 of the BNP sequence. Also they can be used for proBNP immunoassay development in combination with the anti-NT-proBNP MAbs (5B6, 15F11, 11D1) as coating or detection antibodies.

New monoclonal anti-BNP antibody clones and recommendations for sandwich ELISA (monoclonal-monoclonal pairs) will be available in September 2004. Please contact our customer service for additional information.

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
1B6	4BNP2	human BNP, proBNP	IgG2b	EIA, Sandwich immunoassay (capture, detection), WB
2D3	4BNP2	human BNP, proBNP	IgG2a	EIA, Sandwich immunoassay (capture, detection)
2G9	4BNP2	human BNP, proBNP	IgG1	EIA, Sandwich immunoassay (capture, detection)
5F9	4BNP2	human BNP, proBNP	IgG1	EIA, Sandwich immunoassay (capture, detection)
7F5	4BNP2	human BNP, proBNP	IgG1	EIA, Sandwich immunoassay (capture, detection)
10B5	4BNP2	human BNP, proBNP	IgG2b	EIA, Sandwich immunoassay (capture)

2. Anti-BNP polyclonal antibodies

Host animal:	*rabbit*
Antigen:	*synthetic peptide corresponding to peptide 1 – 10 of BNP sequence, conjugated with carrier protein*
Specificity:	*specific to BNP peptide 1 – 10 a.a.r.*
Purification method:	*immunoaffinity chromatography*

Antibodies were generated by animal immunization with peptide corresponding to amino acid residues 1-10 of human BNP sequence, conjugated with carrier protein. Peptide-specific polyclonal antibodies were purified by means of affinity chromatography.

Ordering information

Product	Cat. #	Remarks
Polyclonal anti-BNP antibody, peptide 1-10	**PBNP10**	Available on request

References:
1. *Mair J, Hammerer-Lercher A, Puschendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. Clin Chem Lab Med. 2001 Jul;39(7):571-88.*
2. *Cowie MR, Mendez GF. BNP and congestive heart failure. Prog Cardiovasc Dis. 2002 Jan-Feb;44(4):293-321.*

VI Human N-Terminal proBNP (NT-proBNP)

In cardiac tissue brain natriuretic peptide (BNP) is synthesized as 134 amino acid precursor (prepro-BNP), which is cleaved by proteases to form a 26 a.a.r. "signal" peptide and a 108 a.a.r. pro-BNP. Proteolytic digestion of pro-BNP results in formation of 76 aa amino-terminal NT-proBNP and biologically active 32 aa BNP hormone molecule. Both proBNP and NT-proBNP circulate in human plasma and have been proposed as markers for early diagnosis of left ventricular dysfunction as well as prognostic markers of possible cardiac complications at patients with heart failure.

In early studies it has been demonstrated that NT-proBNP concentration, like BNP concentration, increases in human blood with the severity of congestive heart failure. However it is not yet clearly determined which marker, BNP or NT-proBNP, is superior in the early diagnosis of heart failure. Quantitative measurement of NT-proBNP in blood provides an objective indicator of congestive heart failure severity. Also NT-proBNP is useful as a marker for risk stratification after myocardial infarction. It may be possible that the combination of cardiac troponin and BNP (or NT-proBNP) is the best discriminator for identifying patients who are at the highest risk for adverse events (1-3).

1. Human recombinant NT-proBNP

Source: *E. coli*
Purity: *≥ 95 % according to SDS-PAGE (Fig. 19)*
Application: *NT-proBNP and proBNP immunoassay, immunoblotting*

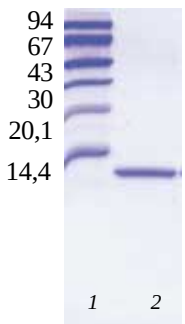


Fig. 19. Tricine-SDS-PAGE of recombinant NT-proBNP in reducing conditions.
Lane 1 - Molecular weight standards (Pharmacia)
Lane 2 - recombinant NT-proBNP (2.5µg)
Gel staining: Coomassie brilliant blue R-250

Ordering information

Product	Cat. #	Purity	Source
Human recombinant NT-proBNP	**8NT1**	>95 %	*E.coli*

2. Anti-NT-proBNP monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp 2/0*
Antigen: *synthetic peptides corresponding to amino acids 1-12, 13-27, 28-45, 46-60, 61-76 of the NT-proBNP sequence, conjugated with carrier protein*
Specificity: *human NT-proBNP and proBNP*
Purification method: *protein A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *NT-proBNP and proBNP immunoassay, NT-proBNP and proBNP immunodetection in Western blotting*

Hybridomas producing MAbs were generated after immunization of Balb/c mice with synthetic peptides, corresponding to different parts of NT-proBNP sequence (Fig. 20), conjugated to carrier protein.

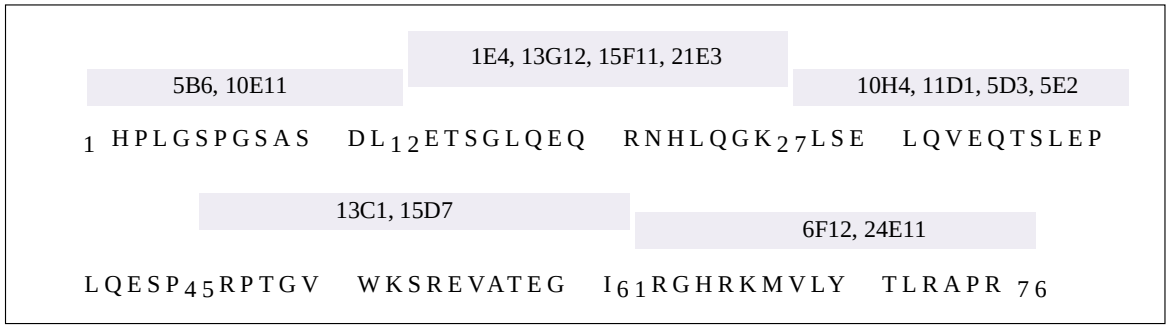


Fig. 20. Primary structure of NT-proBNP and epitope mapping of anti- NT-proBNP antibodies

MAbs presented in this catalogue were selected among antibodies specific to NT-proBNP. All MAbs were tested in sandwich immunoassay as capture and detection antibodies. Different combinations of monoclonal antibodies specific to different epitopes could be used for immunoassay development. The best combinations of antibodies suitable for the development of highly sensitive sandwich immunoassay are:

11D1	5B6
10H4	5B6
5E2	5B6
15F11	5B6
5E2	1E4
5B6	13G12
5E2	13C1
5B6	11D1
5B6	13C1
21E3	11D1
7B5	11D1
15F11	13C1
11D1	13G12
24E11	5B6

Anti-NT-proBNP MAbs can also be used for proBNP immunoassay development in combination with anti–BNP MAbs. For such an assay the best pairs are:

10B5 (anti-BNP)	11D1 (anti-NT-proBNP)
5B6 (anti-NT-proBNP)	5F9 (anti-BNP)

All MAbs recognize NT-proBNP and proBNP also in Western Blotting (Fig. 22).

Fig. 22. Detection of human recombinant NT-proBNP in Western blotting by different monoclonal antibodies after Tricine-SDS gel electrophoresis.

1 - MAb 5B6
2 - MAb 15F11
3 - MAb 11D1
4 - MAb 15D7
5 - MAb 13C1
6 - MAb 24E11

The best pairs demonstrate high sensitivity (20 pg/ml, Fig. 21), low background and high reproducibility. Assays utilizing MAbs 10H4 – 5B6 and 11D1 – 5B6 were tested with blood samples from patients with unstable angina and myocardial infarction.

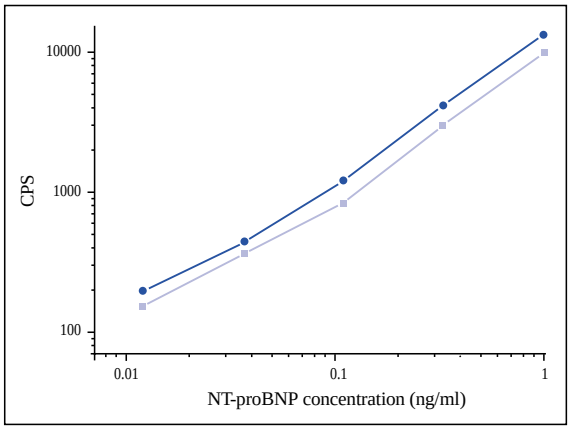
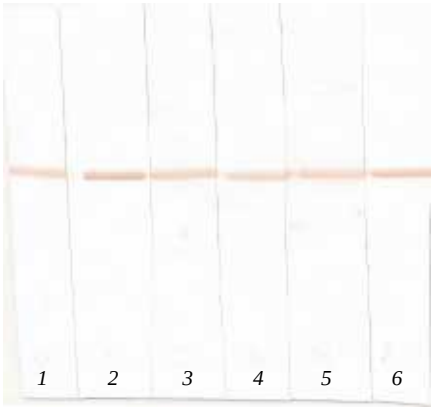


Fig. 21. NT-proBNP calibration curves using pairs 10H4 – 5B6 (-▪-) and 15F11 – 5B6 (-●-).
- capture: 2 µg/well
- detection: Eu-labeled 5B6; 200 ng/well
Antigen: recombinant NT-proBNP
Total incubation time 1 h
Room temperature



Ordering information

Clone	Cat. #	Specificity, a.a.r.	Subclass	Application
5B6	4NT1	1 - 12	IgG1	EIA, Sandwich immunoassay, WB
10E11	4NT1	1 - 12	IgG1	EIA, Sandwich immunoassay, WB
10H4	4NT1	28 - 45	IgG1	EIA, Sandwich immunoassay, WB
11D1	4NT1	28 - 45	IgG1	EIA, Sandwich immunoassay, WB
5D3	4NT1	28 - 45	IgG1	EIA, Sandwich immunoassay, WB
5E2	4NT1	28 - 45	IgG	EIA, Sandwich immunoassay, WB
1E4	4NT1	13 - 27	IgG1	EIA, Sandwich immunoassay, WB
13G12	4NT1	13 - 27	IgG2a	EIA, Sandwich immunoassay, WB
15F11	4NT1	13 - 27	IgG2b	EIA, Sandwich immunoassay, WB
21E3	4NT1	13 - 27	IgG1	EIA, Sandwich immunoassay, WB
13C1	4NT1	46 - 60	IgG1	EIA, Sandwich immunoassay, WB
15D7	4NT1	46 - 60	IgG1	EIA, Sandwich immunoassay, WB
6F12	4NT1	61 - 76	IgG	EIA, Sandwich immunoassay, WB
24E11	4NT1	61 - 76	IgG	EIA, Sandwich immunoassay, WB

New high affinity antibody clones will be available in September 2004. Please contact our customer service for additional information.

3. Anti-NT-proBNP polyclonal antibodies

Host animal: *rabbit*
Antigen: *synthetic peptides corresponding to amino acids 1-12, 13-27, 28-45, 46-60, 61-76 of NT-proBNP sequence, conjugated with carrier protein*
Specificity: *specific to human recombinant NT-proBNP and proBNP (polyclonal antibodies with defined epitope specifity)*
Purification method: *immunoaffinity chromatography*

Antibodies were generated by animal immunization with peptides corresponding to different parts of NT-proBNP sequence. Peptide-specific polyclonal antibodies were purified by means of affinity chromatography.

Ordering information

Product	Specificity, a.a.r.	Remarks
NT-proBNP polyclonal	1 – 12	Available on request
NT-proBNP polyclonal	5 – 20	Available on request
NT-proBNP polyclonal	1 – 24	Available on request
NT-proBNP polyclonal	13 – 27	Available on request
NT-proBNP polyclonal	28 – 45	Available on request
NT-proBNP polyclonal	46 – 60	Available on request
NT-proBNP polyclonal	61 – 76	Available on request

References:
1. *Hobbs FD, Davis RC, Roalfe AK, Hare R, Davies MK, Kenkre JE. Reliability of N-terminal pro-brain natriuretic peptide assay in diagnosis of heart failure: cohort study in representative and high risk community populations. BMJ. 2002 Jun 22;324(7352):1498.*
2. *Pfister R, Scholz M, Wielckens K, Erdmann E, Schneider CA. Use of NT-proBNP in routine testing and comparison to BNP. Eur J Heart Fail. 2004 Mar 15;6(3):289-93.*
3. *Hall C. Essential biochemistry and physiology of (NT-pro)BNP. Eur J Heart Fail. 2004 Mar 15;6(3):257-60.*

VII 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*

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 tissue 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 consists of 132 amino acid residues with 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. Because cFABP concentration is significantly lower in skeletal muscle (comparing with 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 fact 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 of minor myocardial events such as unstable angina. Switching clinical studies from myoglobin to cFABP can be helpful for the improvement of early AMI diagnosis.

FABP 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
Human Fatty Acid Binding Protein (FABP)	**8F65**	>95 %	Human cardiac muscle

2. Anti-FABP monoclonal antibodies

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

Hybridoma cell lines producing anti-FABP MAbs were generated after immunization of Balb/c mice with purified human FABP. The best combination for sandwich immunoassay is MAb 9F3 used as capture antibody and MAb 10E11 as detection antibody (Fig. 23). 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. 24).

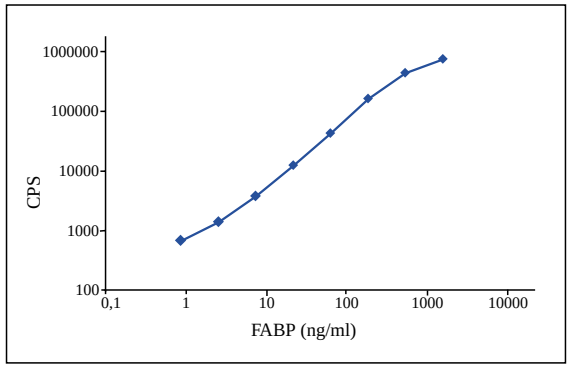


Fig. 23. Calibration curve of FABP sandwich immunoassay.

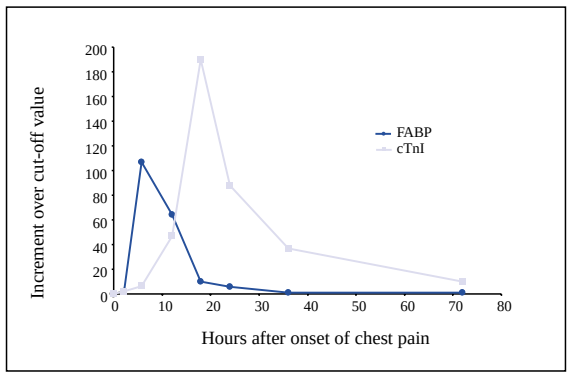


Fig. 24. Time-dependent changes of FABP (dark blue) and cTnI (light blue) concentrations in the blood of representative AMI patient.

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
6B6	4F29	FABP	IgG2b	WB
5B5	4F29	FABP	IgG1	EIA, Sandwich immunoassay
9F3	4F29	FABP	IgG1	EIA, Sandwich immunoassay
10E1	4F29	FABP	IgG1	EIA, Sandwich immunoassay

3. FABP free serum

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

FABP 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.

FABP free serum can be used as a matrix for standard and calibrator preparations. According to ELISA testing FABP free serum does not contain more than 0.5 ng/ml of human FABP.

Ordering information

Product	Cat. #	Source
FABP free serum	**8FFS**	Pooled normal human serum

VIII Glycogen Phosphorylase, BB Isoenzyme (GPBB)

1. Human 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*

Glycogen phosphorylase BB is an enzyme playing an important role in the glycogen turnover. Three known isoforms of glycogen phosphorylase 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) and 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 myocardial cell death and its 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 e.g. in patients with bypass surgery and unstable angina. All these features make GPBB a very promising marker of myocardial cell injury, but its cardio-specificity should still be estimated.

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 glycogen phosphorylase MM isoenzyme.

Ordering information

Product	Cat. #	Purity	Source
Human 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 to the BB isoenzyme, others recognize MM isoenzyme as well. The best combinations to be used in sandwich immunoassay are:

1G6 —— 6F1
1G6 —— 9F5
6F1 —— 7B9
17B6 —— 10H5

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
7B9	4GP31	BB isoenzyme	IgG1	EIA, Sandwich immunoassay (detection), WB
8G7	4GP31	BB isoenzyme	IgG1	WB
6G5	4GP31	BB isoenzyme	IgG1	WB
11C10	4GP31	BB isoenzyme	IgG1	WB
1G6	4GP31	BB isoenzyme	IgG2b	EIA, Sandwich immunoassay (capture), WB
17B6	4GP31	BB and MM isoenzyme	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
10D12	4GP31	BB and MM isoenzyme	IgG1	WB
6F1	4GP31	BB and MM isoenzyme	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
10H5	4GP31	BB and MM isoenzyme	IgG2a	EIA, Sandwich immunoassay (detection), WB
5B8	4GP31	BB and MM isoenzyme	IgG1	WB
3G1	4GP31	BB and MM isoenzyme	IgG1	WB
9F5	4GP31	BB and MM isoenzyme	IgG1	EIA, Sandwich immunoassay (detection), WB

IX Human C-Reactive Protein (CRP)

1. Human 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, immunological and mass CRP standard, CRP biochemical and immunochemical studies*

CRP – "acute phase serum protein" is known for several decades as a non-specific inflammation marker. Recent studies revealed that among other markers of inflammation, CRP shows the strongest association with cardiovascular events. Many clinical studies demonstrated that coronary mortality among patients with unstable angina and elevated CRP is significantly higher comparing with the patients without elevated CRP. These data suggest that the intensity of the vascular inflammatory process is a determinant of clinical outcome in unstable coronary artery disease.

Measurements of C-reactive protein (hsCRP) in the patients with ischemic heart disease provide a novel method for detecting individuals at high risk of plaque rapture. This new clinical application requires CRP quantification in the sample in the concentrations below those traditionally measured by the majority of the commercially available assays. New type of CRP assays - high sensitivity CRP (hsCRP) assays with the detection limit 0.1 - 0.2 µg/ml are widely used in clinical practice for the discrimination of patients with elevated risk of cardiac complications.

Ordering information

Product	Cat. #	Purity	Source
Human C-Reactive Protein	**8C72**	>95 %	Human pleural/ascitic fluid or plasma

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, CRP immunodetection in Western blotting, CPR immunoaffinity purification*

Hybridoma clones producing anti-CRP MAbs were generated after immunization of Balb/c mice with purified human CRP. Four MAbs (C2, C5, C6 and C7) recognize antigen both in the presence and in the absence of Ca^{2+} (samples containing EDTA, Fig. 26). MAbs C1, C3 and C4 do not interact with Ca^{2+}-depleted protein. Best combinations for the development of sandwich immunoassay are:

C2 ——— C6 (Fig. 25)
C5 ——— C6
C7 ——— C6
C5 ——— C2
C5 ——— C7

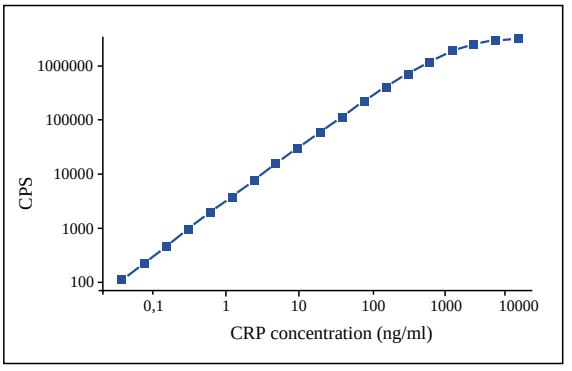


Fig. 25. Calibration curve for C2-C6 sandwich immunoassay. MAb C2 is biotinylated, MAb C6 is labeled with stable Eu^{3+} chelate. Mixture of antibodies and antigen samples (10 µL) was incubated for 10 min at room temperature in streptavidin coated plates.

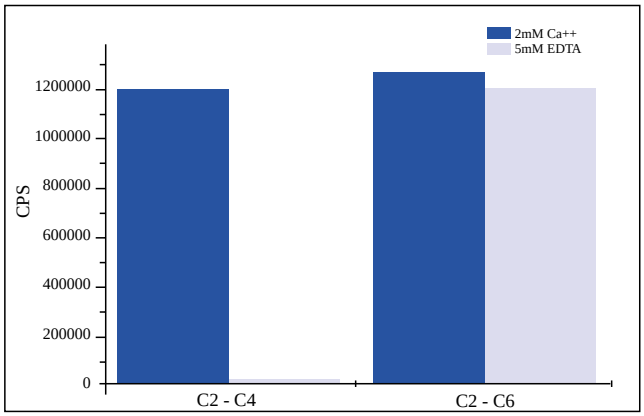


Fig. 26. Sensitivity to EDTA. Serum samples with (dark blue) and w/o (light blue) Ca^{2+} ions were tested by two two-site combinations of RDI anti-CRP MAbs. MAb C4 is sensitive to the conformational changes of CRP molecule and thus the assay gives no signal in the presence of EDTA. Sandwich immunoassay utilizing MAbs C2 and C6 recognizes the antigen with the same efficiency in the presence and in the absence of EDTA.

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
C1	4C28	CRP	IgG2b	WB
C2	4C28	CRP	IgG1	EIA, Sandwich immunoassay (capture, detection)
C3	4C28	CRP	IgG1	EIA, Sandwich immunoassay
C4	4C28	CRP	IgG1	EIA, Sandwich immunoassay, WB
C5	4C28	CRP	IgG1	EIA, Sandwich immunoassay (capture)
C6	4C28	CRP	IgG2a	EIA, Sandwich immunoassay (detection)
C7	4C28	CRP	IgG1	EIA, Sandwich immunoassay (capture, detection)

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. According to ELISA testing the level of the antigen in CRP free serum is below 0.02 ng/ml (Fig. 27).

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

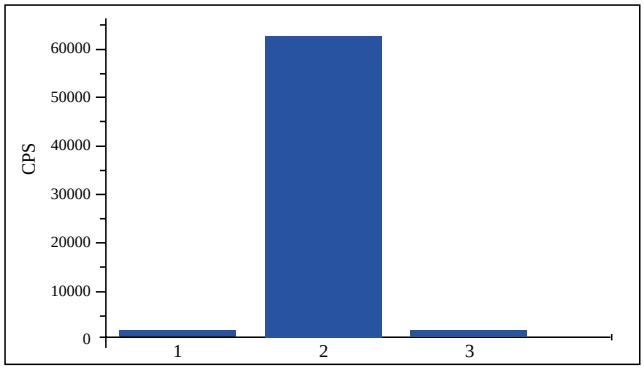


Fig. 27. CRP level in normal human serum and in CRP free serum detected in sandwich-immunoassay.
1 - Buffer
2 - Initial signal in normal human serum 25 fold diluted (corresponds to 4 µg/ml of CRP)
3 - Residual signal in CRP free serum

Ordering information

Product	Cat. #	Source
CRP Free serum	**8CFS**	Pooled normal human serum

X Myeloperoxidase (MPO)

1. Human MPO

Source: *human leukocyte mass*
Purity: *> 85 % by SDS-PAGE*
Presentation: *lyophilized*
Application: *immunological and mass MPO standard, immunogen for antibody production, MPO enzymatic, biochemical and immunochemical studies*

Myeloperoxidase is a hemoprotein that is abundantly expressed in neutrophils and secreted during their activation. Native MPO is represented as a covalently bound tetrameric complex of two glycosylated alpha chains (MW 59 - 64 kDa) and two unglycosylated beta chains (MW 14 kDa) with total MW 150 kDa and theoretical pI 9.2 (1).

Traditionally MPO was considered as a main target of anti-neutrophil cytoplasm antibodies (ANCA), the serological markers for certain systemic vasculities e.g. periarteriitis nodosa, microscopic polyarteriitis and pulmonary eosinophilic granulomatosis (Churg-Strauss syndrome) (2). Low to moderate anti-MPO autoantibody levels are also reported in rheumatoid arthritis.

Recently it was shown that MPO participates in the initiation and progression of cardiovascular disease. It possesses potent proinflammatory properties and may contribute directly to tissue injury. Now MPO is under consideration as one of the most promising cardiac markers because initial measurement of serum MPO level predicts the early risk of myocardial infarction, as well as the risk of major adverse cardiac events in the ensuing 30-day and 6-month periods. In addition, MPO predicts these outcomes independently without other known laboratory tested risk factors, including troponins, CK-MB, CRP and lipid profile. MPO levels, in contrast to troponins I and T, creatine kinase MB isoform, and C-reactive protein levels, identify patients at risk for cardiac events in the absence of myocardial necrosis. It means that MPO level measurement may be the only way to identify patients with chest pain that are at increased risk of cardiovascular events, without invasive diagnostic testing (3, 4).

On gel after protein separation by SDS-PAGE in reducing conditions MPO is presented by two main bands with apparent molecular masses 60 kDa and 14 kDa and with minor contamination band of 40 kDa (Fig. 28)

Fig. 28. SDS-PAGE of human MPO in reducing conditions.
Lane 1 - Molecular weight standards (Pharmacia)
Lane 2 - Human MPO 4µg

Ordering information

Product	Cat. #	Purity	Source
Human myeloperoxidase	**8M80**	>85 %	Human leukocyte mass

2. Anti-MPO monoclonal antibodies

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

Hybridomas producing MAbs were generated after Balb/c mice immunization with purified human MPO. Different combinations of monoclonal antibodies could be used for immunoassay development. The best combinations for sandwich immunoassay are:

4A4 —— 2A11
2A11 —— 4B2

These MAb pairs were tested with serum samples of AMI patients. MAb 4A4 can also be used for MPO immunodetection in Western Blotting.

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
4F10	4M43	MPO	IgG2a	EIA, Sandwich immunoassay (detection)
4B2	4M43	MPO	IgG2a	EIA, Sandwich immunoassay (detection)
4A6	4M43	MPO	IgG2b	EIA, Sandwich immunoassay (capture)
3B11	4M43	MPO	IgG2a	EIA, Sandwich immunoassay
2A11	4M43	MPO	IgG2a	EIA, Sandwich immunoassay (capture)
4A4	4M43	MPO	IgG2b	EIA, Sandwich immunoassay (detection), WB

3. MPO free serum

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

MPO free serum is prepared from pooled normal human serum by immunoaffinity chromatography method. The affinity sorbent utilizes several MAbs with different epitope specificity.

MPO free serum can be used as a matrix for standard and calibrator preparation.

Ordering information

Product	Cat. #	Source
MPO free serum	**8MPFS**	Pooled normal human serum

References:
1. *Nauseef WM, Olsson I, Arnljots K.(1988) Biosynthesis and processing of myeloperoxidase – a marker for myeloid cell differentiation. Eur J Haematol, 40(2): 97-110*
2. *Choi HK, Liu S, Merkel PA, Colditzga, Niles JL. (2001) Diagnostic performance of antineutrophil cytoplasmic antibody tests for idiopathic vasculitides: metaanalysis with a focus on antimyeloperoxidase antibodies. J Rheumatol. 28(7): 1584-1590*
3. *Brennan ML et al. (2003) Prognostic value of myeloperoxidase in Patients with chest pain. New Eng J Med,, 349(17): 1595-1604*
4. *Baldus S. et al. (2003) Myeloperoxidase serum level predicts risk in patients with acute coronary syndromes. Circulation 108: 1440-1445.*

XI D-Dimer and High Molecular Weight Fibrin Degradation Products

1. Human D-Dimer

Source: *human plasma*
Purity: *> 90 % by SDS-PAGE*
Presentation: *lyophilized*
Application: *immunological D-Dimer standard, biochemical and immunochemical studies*

Fibrinogen is the main protein of blood coagulation system. It consists of two identical subunits that contain three polypeptide chains: α, β and γ. The process of blood coagulation results in the activation of fibrinogen into fibrin by thrombin and fibrin polymerization. Fibrin clot is then digested by plasmin, and fibrin degradation products of different molecular weights are released into the bloodstream.

D-dimer is the main (smallest) product of fibrin degradation (MW 180 kDa, Fig. 29). It consists of 111 – 197 amino acids of α-chain, 134 – 461 amino acids of β-chain, and 88 – 406 amino acids of γ-chain of fibrinogen. All chains are cross-linked by disulfide bonds, and the dimeric structure is held by two iso-peptide bonds between C-terminal parts of γ-chains.

D-dimer level in healthy individuals is less than 0.5 μg/ml. Elevated level of D-dimer was found in the blood of patients with pulmonary thromboembolism, deep vein thromboses, atherosclerosis and other cardio-vascular diseases. Elevated level of D-dimer in blood indicates a risk of myocardial infarction and is believed to be a reliable marker of pathological coagulation that underlies pathogenesis of most cardiovascular diseases (1, 2).

RDI offers D-dimer produced from clotted fibrinogen by means of plasmin digestion.

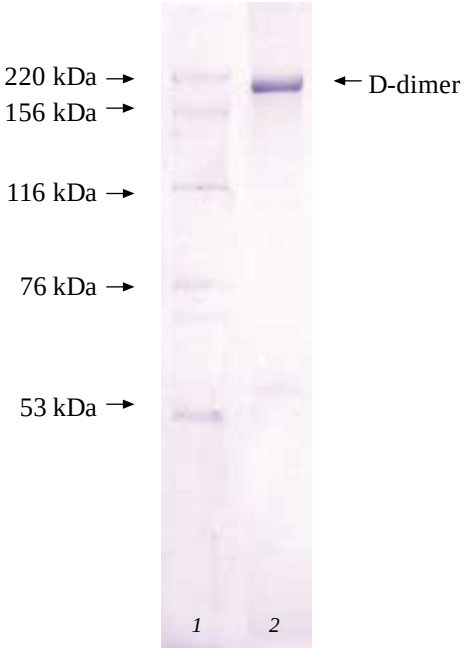


Fig. 29. SDS gel electrophoresis of D-dimer under non-reducing conditions.
Lane 1 - molecular weight standards
Lane 2 - D-dimer (3 μg)
Gel staining: Coomassie brilliant blue R-250

Ordering information

Product	Cat. #	Purity	Source
Human D-Dimer	**8D70**	>90 %	Human plasma

2. Anti-D-Dimer monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *fibrin clot*
Purification method: *protein A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *D-Dimer immunoassay, cross-linked fibrin degradation product and D-Dimer immunodetection in Western Blotting.*

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with homogenized fibrin clot.

RDI offers:

A. Antibodies specific to D-dimer that do not show cross-reactivity with fibrinogen or D-monomer (Fig. 30):

DD1 and DD3 – specific to D-dimer and high molecular weight fibrin degradation products
DD2 – more specific to high molecular weight fibrin degradation products than to D-dimer

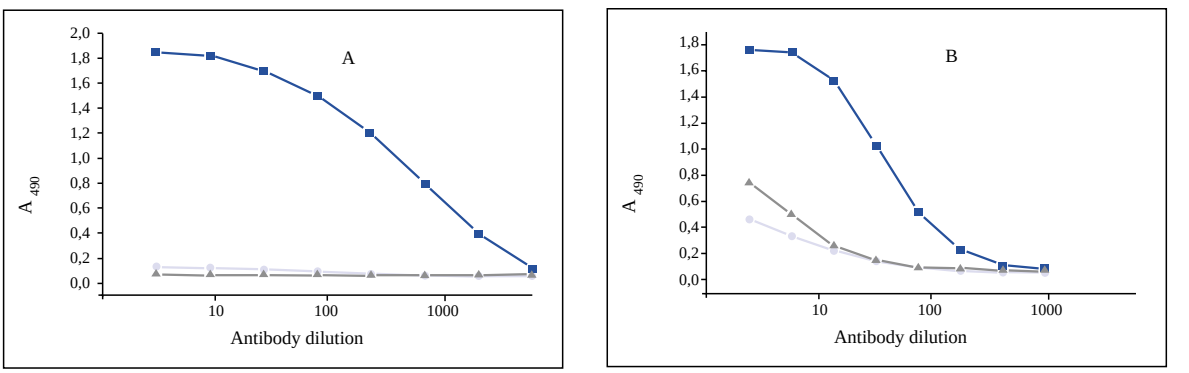


Fig. 30. Titration curves of D-dimer specific monoclonal antibodies (A – DD1, B – DD2).
Antigen for coating: 200 ng/well (■ - D-dimer, ▲- fibrinogen, ● - D-monomer)
Initial DD1 and DD2 antibody concentration: 100 μg/ml
Comments: In case fibrinogen is used for plate coating, both MAbs recognize high molecular weight fibrin degradation products presented in protein preparation as contaminant material.

Specificity of anti-D-dimer antibodies was studied in Western blotting. As seen in Fig. 31, MAb DD1 recognizes D-dimer and does not recognize fibrinogen, D-monomer or D-dimer treated with mercaptoethanol.

Fig. 31. D-dimer, D-monomer and fibrinogen immunodetection by MAb DD1 in Western blotting
Lane 1 - D-dimer
Lane 2 - D-monomer
Lane 3 - fibrinogen
Lane 4 - D-dimer treated with mercaptoethanol

B. Antibodies specific to D-dimer and high molecular weight fibrin degradation products, which show cross-reactivity with fibrinogen or D-monomer.

DD4, DD5 and DD6 – specific to D-dimer and high molecular weight fibrin degradation products, low cross-reaction with D-monomer, cross-reaction with fibrinogen.

DD9 and DD10 – specific to D-dimer and high molecular weight fibrin degradation products, cross-reaction with D-monomer and fibrinogen.

Recommended pairs to be used in sandwich immunoassay for D-dimer detection in human plasma:

DD1 —— DD6
DD1 —— DD4
DD1 —— DD5
DD3 —— DD4

Recommended pairs to be used in sandwich immunoassay for detection of high molecular weight fibrin degradation products in human plasma:

DD2 —— DD6
DD2 —— DD4
DD2 —— DD5

The recommended pairs are specific to cross-linked material and do not show cross-reactivity with fibrinogen, fibrinogen degradation products and D-monomer.

Because of cross-reaction of all detection antibodies with fibrinogen we strongly recommend to use a two-step incubation procedure. On the first step incubate capture antibodies with plasma samples and on the second incubate the immune complex (capture MAb - antigen) with the detection antibodies to form a sandwich.

To be analyzed in sandwich immunoassay, plasma must be diluted with 10 mM Tris-HCl buffer, pH 7.5, containing 1 M NaCl and 0.1 % Tween 20. To avoid non-specific binding the final NaCl concentration in plasma samples must be 0.5 M or more.

Less than 10 ng/ml of D-dimer can be detected by DD1 – DD6 assay using ordinary ELISA approach with HRP-labeled DD6 MAb.

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
DD1	4D30	No C/R with D-monomer and fibrinogen	IgG2a	EIA, Sandwich immunoassay (capture), WB
DD2	4D30	No C/R with D-monomer and fibrinogen	IgG2b	EIA, Sandwich immunoassay (capture), WB
DD3	4D30	No C/R with D-monomer and fibrinogen	IgG2b	EIA, Sandwich immunoassay (capture), WB
DD4	4D30	Low C/R with D-monomer,		
		C/R with fibrinogen	IgG2b	EIA, Sandwich immunoassay (detection), WB
DD5	4D30	Low C/R with D-monomer,		
		C/R with fibrinogen	IgG2b	EIA, Sandwich immunoassay (detection), WB
DD6	4D30	Low C/R with D-monomer,		
		C/R with fibrinogen	IgG2a	EIA, Sandwich immunoassay (detection), WB
DD9	4D30	C/R with D-monomer and fibrinogen	IgG2b	EIA, Sandwich immunoassay, WB
DD10	4D30	C/R with D-monomer and fibrinogen	IgG2a	EIA, Sandwich immunoassay, WB

References:
1. *Bounameaux H, de Moerloose P, Perrier A, Reber G. Plasma measurement of D-dimer as diagnostic aid in suspected venous thromboembolism: an overview. Thromb Haemost. 1994; 71:1-6.*
2. *Rowbotham BJ, Carroll P, Whitaker AN, Bunce IH, Cobcroft RG, Elms MJ, Masci PP, Bundesen PG, Rylatt DB, Webber AJ. Measurement of crosslinked fibrin derivatives--use in the diagnosis of venous thrombosis. Thromb Haemost. 1987; 57:59-61.*

XII Thrombin Activatable Fibrinolysis Inhibitor (TAFI)

1. Human TAFI

Source: *human plasma*
Purity: *> 95 % by SDS-PAGE*
Presentation: *solution of frozen*
Application: *immunological and mass TAFI standard*

Thrombin activatable fibrinolysis inhibitor (TAFI; synonyms: procarboxypeptidase B, procarboxypeptidase U, procarboxypeptidase R) belongs to a family of Zn-containing metallocarboxypeptidases specific to C-terminal lysine and arginine residues. It circulates in plasma as a zymogen with molecular weigh of 55 kDa (401 amino acid residues; pI 5.0, Fig. 32). Being activated by thrombin-thrombomodulin complex during blood coagulation, it exerts carboxypeptidase activity. Activated TAFI removes C-terminal lysine residues from fibrin, which are necessary for plasminogen binding to fibrin. This prevents plasminogen from activation into plasmin and retards the lysis of a fibrin clot.

The concentration of TAFI in plasma of healthy people is 5 – 10 µg/ml. High plasma levels of TAFI were found in patients with stable angina pectoris and angiographically verified coronary artery disease. Elevated TAFI concentration in blood is considered as a risk factor for venous thrombosis. A deficiency of TAFI might contribute to the severity of bleeding disorders in hemophilias A and B. The TAFI level is decreased in chronic liver disease. All these facts make TAFI an important diagnostic parameter in cardiovascular diseases.

RDI's TAFI is purified from human plasma by affinity chromatography.

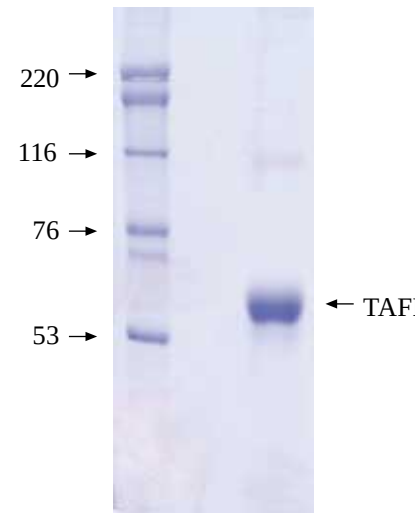


Fig. 32. SDS gel electrophoresis of TAFI in non-reducing conditions.
Lane 1 - molecular weight standards
Lane 2 - human TAFI (5 µg)
Gel staining: Coomassie brilliant blue R-250

Ordering information

Product	Cat. #	Purity	Source
Human TAFI	**8TA1**	>95 %	Human plasma

2. Anti-TAFI monoclonal antibodies

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

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with purified human TAFI. Specificity of antibodies was confirmed by ELISA and Western Blotting. All antibodies recognize TAFI in ELISA (Fig. 34). MAbs 13H4, 16C5 and 19E2 recognize TAFI in Western Blotting after SDS gel electrophoresis under non-reducing conditions (Fig. 33).

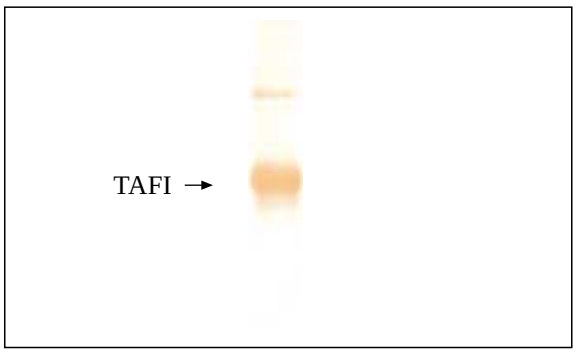
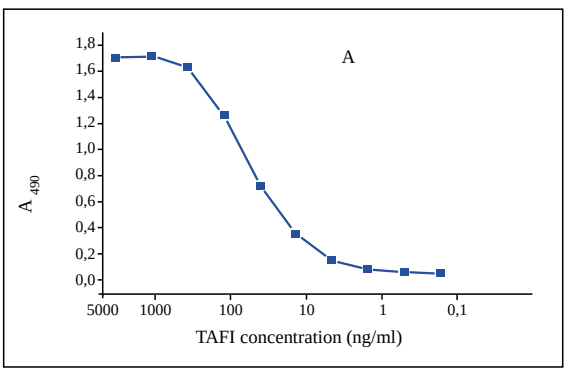


Fig. 33. Detection of TAFI by MAb 13H4 in Western Blotting (non-reducing conditions).



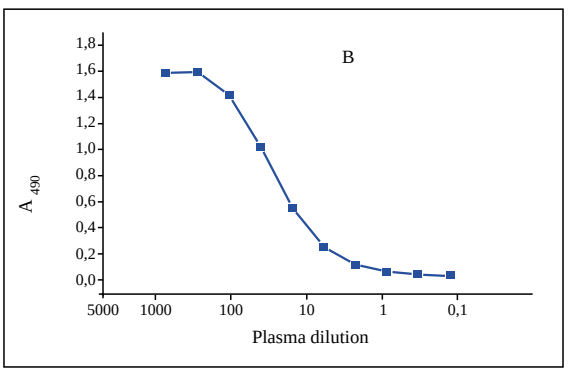


Fig. 34. Titration curves of purified TAFI (A) and TAFI in plasma (B) using antibody pair 13D5 – 13H4.
Capture: MAb 13D5; 200 ng/well
Detection: MAb 13H4 conjugated with HRP
Room temperature

Recommended pairs to be used for TAFI detection in human plasma by sandwich immunoassay:

13D5 —— 13H4
13D5 —— 19E2
15A10 —— 13H4
15A10 —— 19E2
16C5 —— 13D5

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
13C3	4TA1	TAFI	IgG2a	EIA, Sandwich immunoassay (capture, detection)
13D5	4TA1	TAFI	IgG2a	EIA, Sandwich immunoassay (capture, detection)
13H4	4TA1	TAFI	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
15A10	4TA1	TAFI	IgG2a	EIA, Sandwich immunoassay (capture, detection)
19E2	4TA1	TAFI	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
16C5	4TA1	TAFI	IgG1	EIA, Sandwich immunoassay (capture, detection), WB

References:
1. Van Tilburg N.H. et al. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 2000; 95: 2855-9.
2. Silveira A. et al. Plasma procarboxypeptidase U in men with symptomatic coronary artery disease. Thromb Haemost 2000; 84: 364-8.
3. Schroeder V. et al. Thrombin activatable fibrinolysis inhibitor (TAFI) levels in patients with coronary artery disease investigated by

XIII Fibrinogen

Fibrinogen is the main protein of blood coagulation system. It is a large protein (MW 340 kDa) and it consists of two identical subunits that contain three polypeptide chains: α, β and γ. All chains are connected with each other by a number of disulfide bonds. Fibrinopeptides A (1 – 16 amino acids) and B (1 – 17 amino acids) are released by thrombin from the N-terminal parts of α- and β-chains, respectively. In this way fibrinogen is converted into fibrin, which by means of polymerization forms a fibrin clot. Fibrinogen clotting underlies pathogenesis of MI, thromboembolism and thromboses of arteries and veins, since fibrin is the main substrate for thrombus formation. Fibrinogen activation is also involved in pathogenesis of inflammation, tumor growth and many other diseases.

The normal fibrinogen concentration in plasma is about 3 mg/ml. The elevated level of fibrinogen in patient's blood is regarded as an independent risk factor for cardiovascular diseases. An increase in blood fibrinogen concentration was shown to be a strong predictor of coronary heart disease (1, 2). All these facts make fibrinogen an important parameter in the diagnosis of cardiovascular diseases.

1. Anti-fibrinogen monoclonal antibodies

Host animal: *mice BALB/c*
Cell line used for fusion: *Sp2/0*
Antigen: *Fibrinopeptide A or human fibrin degradation products*
Purification method: *protein A affinity chromatography*
Presentation: *MAb solution in PBS with 0.1 % sodium azide*
Application: *Fibrinogen immunoassay and Fibrinogen immunodetection in Western Blotting.*

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with purified human fibrin degradation products or fibrinopeptide A.

Specificity of antibodies was confirmed by ELISA and Western Blotting. All antibodies recognize fibrinogen in ELISA. MAbs 1F3, 6G12, 15E11, 27C8 and 40F11 recognize fibrinogen in Western Blotting after SDS gel electrophoresis under non-reducing conditions; MAbs 15H12 and 41D9 recognize fibrinogen in Western blotting after electrophoresis under both reducing and non-reducing conditions (Fig. 35).

Antibodies recommended for sandwich immunoassay to detect fibrinogen together with fibrin degradation products are:

1F3 —— 27C8
40F11 —— 1F3

For specific fibrinogen detection see section XIV (fibrinopeptide A).

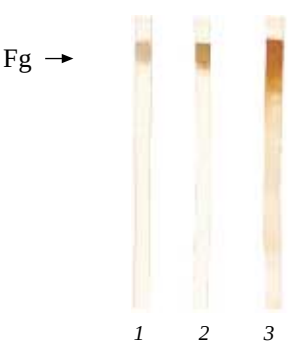


Fig. 35. Detection of fibrinogen by anti-fibrinogen antibodies in Western Blotting (non-reducing conditions). Lane 1 - MAb 1F3; Lane 2 - MAb 6G12; Lane 3 - MAb 15H12

Ordering information

Clone	Cat.#	Specificity	Subclass	Application
1F3	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
6G12	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
15E11	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
15H12	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
27C8	4F1	Fibrinogen, Fibrin degradation products	IgG2a	EIA, Sandwich immunoassay (capture, detection), WB
40F11	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
41D9	4F1	Fibrinogen, Fibrin degradation products	IgG2a	EIA, Sandwich immunoassay (capture, detection), WB

References:
1. Lowe G. et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study. Thromb Haemost, 2000; 84:553-8.
2. Danesh J. et al. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA, 1998; 279:1477-82.

XIV Fibrinopeptide A

Fibrinogen consists of two identical subunits that contain three polypeptide chains: α, β and γ. The process of fibrinogen clotting begins with removal of N-terminal peptides from its α-chains (fibrinopeptide A) and β-chains (fibrinopeptide B) by thrombin that is formed by a set of cascade reactions. The increase of fibrinopeptide A level is a direct marker of fibrinogen clotting. Elevated levels of fibrinopeptide A were found in patients with myocardial infarction, coronary heart disease and other disorders accompanied by the activation of blood coagulation system.

Fibrinogen can be specifically detected by immunological methods only if capture antibodies recognize fibrinopeptides A or B in intact fibrinogen. Otherwise fibrin degradation products will be measured together with fibrinogen. It is not important when fibrinogen is measured in healthy individuals or in patients with mild hypercoagulation but becomes of great significance in disorders with disseminated intravascular coagulation (DIC) when the amount of fibrin degradation products in blood is comparable with the amount of fibrinogen.

1. Synthetic fibrinopeptide A

Source: *amino acid synthesis*
Purity: *> 99 % by SDS-PAGE*
Presentation: *lyophilized*
Application: *immunoassay standard and calibrator*

Fibrinopeptide A is synthesized from amino acids according to the sequence ADSGEGDFLAE GGGVR that is 1–16 amino acids of the α-chain of fibrinogen.

The identity and purity of fibrinopeptide A was confirmed by mass spectroscopy. The purity was found to be more than 99 %.

Ordering information

Product	Cat. #	Purity	Source
Synthetic fibrinopeptide A	**8FP1**	>99 %	Amino acid synthesis

2. Anti-fibrinopeptide A monoclonal antibodies

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

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with fibrinopeptide A conjugated with a carrier protein. Antibodies recognize free fibrinopeptide A and fibrinopeptide A region of fibrinogen α-chain.

For specific fibrinogen detection by sandwich immunoassay we recommend using anti-fibrinopeptide A MAbs as capture and anti-fibrinogen MAbs as detection. Recommended pairs:

49D2 (anti-fibrinopeptide A) — 40F11 (anti-fibrinogen)
1F7 (anti-fibrinopeptide A) — 1F3 (anti-fibrinogen)
26E7 (anti-fibrinopeptide A) — 27C8 (anti-fibrinogen)

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
1F7	4FP1	Fibrinopeptide A	IgG2a	EIA, Sandwich immunoassay (capture)
26E7	4FP1	Fibrinopeptide A	IgG2b	EIA, Sandwich immunoassay (capture)
49D2	4FP1	Fibrinopeptide A	IgG2a	EIA, Sandwich immunoassay (capture)

1. *Sonel A. et al. Prospective Study Correlating Fibrinopeptide A, Troponin I, Myoglobin, and Myosin Light Chain Levels With Early and Late Ischemic Events in Consecutive Patients Presenting to the Emergency Department With Chest Pain. Circulation. 2000; 102:1107-13.*
2. *Rapold H.J. et al. Fibrin formation and platelet activation in patients with myocardial infarction and normal coronary arteries. Eur Heart J. 1989; 10:323-33.*

XV Leptin

One of the most often encountered risk factors of cardiovascular disorders in developed countries is obesity (1). The link between obesity and cardiovascular disease is very complex. Obesity is associated with high insulin and leptin levels. Abnormalities in leptin homeostasis have been proposed to increase the propensity to obesity. Recent studies demonstrated that high serum levels of leptin were an independent risk factor for cardiovascular thrombotic events, such as heart attacks and strokes (2).

Leptin is expressed and secreted by adipose tissue cells, and the level of circulating leptin is directly proportional to the total amount of fat in the body. Leptin exerts an influence in many physiological processes, including food intake, thermoregulation, fertility, thyroid function, adrenal function, sympathetic nerve activation, renal function, blood vessel tone and blood pressure.

Amino acid sequence of human leptin is 85 % identical to mouse protein and 84 % identical to rat leptin. Mouse and rat leptin demonstrate 96 % identity with each other (3, 4). Human leptin is a 146 amino acid residue, 16 kDa, non-glycosylated protein.

1. Anti-leptin monoclonal antibodies

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

Hybridomas producing MAbs were generated after immunization of Balb/c mice with human recombinant leptin. Different combinations of monoclonal antibodies could be used for immunoassay development.

All leptin specific antibodies were tested in sandwich immunoassay as capture and detection antibodies. All MAbs recognize antigen in human blood with high sensitivity. The best pairs recommended for sandwich immunoassay:

3G7 —— 4F12
4F12 —— 3G7
3G7 —— 9C10
4F12 —— 10H1

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
3G7	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (capture, detection)
4F8	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (detection)
4F12	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (capture, detection)
5F10	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (capture, detection)
9C10	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (detection)
10H1	2LE1	Leptin	IgG1	EIA, Sandwich immunoassay (detection)

References:
1. *El-Atat F et al. (2003) Obesity and hypertension. Endocrinol Metab Clin North Am. 32(4):823-854.*
2. *Rahmouni K, Haynes WG. (2004) Leptin and the cardiovascular system. Recent Prog Horm Res. 59:225-244.*
3. *Zhang Y. et al. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425-432.*
4. *Ogawa Y. et al. (1995) Molecular cloning of rat obese cDNA and augmented gene expression in genetically obese Zucker fatty (fa/fa) rats. J Clin Invest 96: 1647-1652.*

XVI Urinary Albumin

Microalbuminuria (an increased urinary albumin excretion greater or equal to 15 µg/min that is not detectable by usual dipstick methods for macro-proteinuria) predicts cardiovascular events in essential hypersensitive patients, yet the pathophysiological mechanisms underlying this association remain to be elucidated (1).

Hypersensitive 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 (2). 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.

Microalbuminuria is recognized as a predictor of subsequent progression of nephropathy, a kidney disease common among people with diabetes. However, in some cases the possibility of non-diabetic nephropathy also needs to be considered. In its early stages, nephropathy is characterized by microalbuminuria that, if left untreated, can lead to serious complications such as kidney failure (3).

Because the albumin molecule is relatively small and albumin's concentration in human blood is very high, it is often among the first proteins to enter the urine after glomeruli are damaged. Therefore, even minor kidney dysfunction is detectable with proper diagnosis of microalbuminuria. Because transient increases in microalbumin can be due to urinary tract infection, exercise, intercurrent illness etc., a first abnormal result should be followed by others over a 2 – 12 month period.

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:	*albumin immunoassay*

Hybridomas producing MAbs were generated after immunization of Balb/c mice with human serum albumin. Different combinations of monoclonal antibodies could be used for immunoassay development.

All albumin-specific antibodies were tested in sandwich immunoassay as capture and detection antibodies. All MAbs recognize antigen in human blood with high sensitivity. There is no cross-reactivity with other human proteins tested or with bovine serum albumin and egg white albumin.

The recommended pairs for immunoassay are:

15C7 —— 1A9
15C7 —— 6B11

RDI's new high affinity anti-human albumin monoclonal antibodies are suitable for the development of sensitive sandwich immunoassay with rapid kinetics (10 – 15 min in plate format) for the detection of albumin in human urine. Antibodies 6B11, 1A9 and 15C7 can be used for the detection of human serum albumin in Western blotting after native gel electrophoresis (Fig. 36). Mab 6B11 recognises albumin from normal human serum in blotting after protein separation by SDS-PAGE in non-reducing conditions.

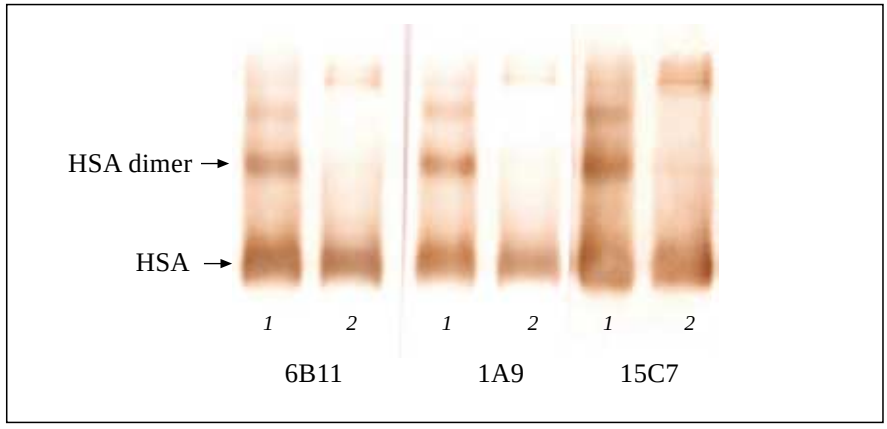


Fig. 36. Detection of human serum albumin by anti-albumin antibodies in Western Blotting.
1 - human serum albumin (Sigma A-1653), 3 µg per track
2 - normal human serum, about 3 µg of human serum albumin per track

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
1C8	4T24	Human serum albumin	IgG1	EIA, Sandwich immunoassay (capture, detection)
1A9	4T24	Human serum albumin	IgG2a	EIA, Sandwich immunoassay (detection), WB
6B11	4T24	Human serum albumin	IgG2a	EIA, Sandwich immunoassay (capture, detection), WB
15C7	4T24	Human serum albumin	IgG2b	EIA, Sandwich immunoassay (capture, detection), WB

References:
1. *Wachtell K et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med. 2003, 139(11), pp.901-906.*
2. *Salmasi AM et al. The degree of albuminuria is related to left ventricular hypertrophy in hypertensive diabetics and is associated with abnormal left ventricular filling: a pilot study. Angiology. 2003, 54(6), pp. 671-678.*
3. *Bennett PH et al. Screening and management of microalbuminuria in patients with diabetes mellitus: recommendations to the Scientific Advisory Board of the National Kidney Foundation from an ad hoc committee of the Council on Diabetes Mellitus of the National Kidney Foundation. Am J Kidney Dis. 1995, 25(1), pp.107-112*

XVII Procalcitonin (PCT)

Procalcitonin (PCT) is a 116 amino acid residue peptide with molecular weight of about 13 kDa. The amino acid sequence of PCT was firstly described by Moullec et al. in 1984 (1) (Fig. 37.). It belongs to a group of related proteins including calcitonin gene-related peptides I and II, amylin, adrenomodulin and calcitonin (CAPA peptide family). PCT, like other peptides of CAPA family, appears from the common precursor pre-procalcitonin consisting of 141 amino acids by removal of 25 a.a.r. from N-terminus.

PCT is produced normally in C-cells of the thyroid glands. It undergoes successive cleavages to form three molecules: N-terminal fragment (55 a.a.r.), calcitonin (32 a.a.r.) and katacalcin (21 a.a.r.).

It has been shown that the level of PCT in serum increases significantly during an infection of bacterial origin (2). Today PCT is considered to be one of the earliest and most specific markers of sepsis. However, several studies revealed that elevated PCT level in human blood could be detected not only in case of sepsis and infection, but also in cases of surgery, polytrauma, heat shock and cardiogenic shock (3). The importance of PCT measurements in combination with cTnT or cTnI during heart transplantation to predict an early graft failure has been proved recently (4).

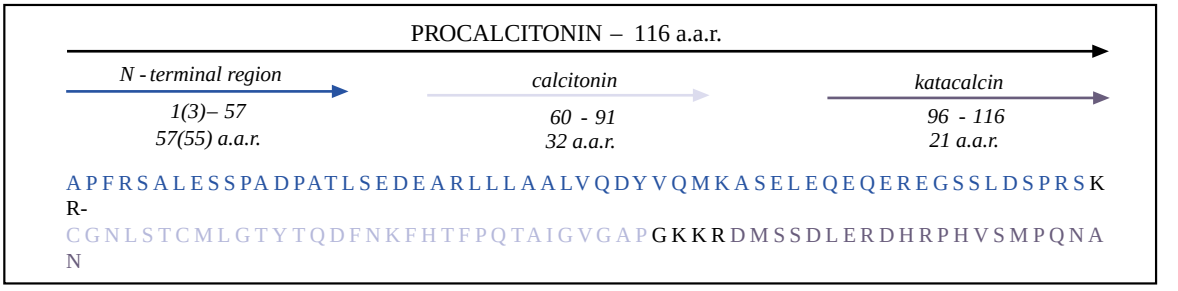


Fig. 37. Amino acid sequence and schematic diagram of human procalcitonin.

1. Anti-procalcitonin monoclonal antibodies

Host animal:	*mice BALB/c*
Cell line used for fusion:	*Sp2/0*
Antigen:	*fragments of procalcitonin, conjugated with carrier protein*
Purification method:	*protein A affinity chromatography*
Presentation:	*MAb solution in PBS with 0.1 % sodium azide*
Application:	*procalcitonin immunoassay*

Hybridomas producing anti-PCT MAbs were generated after immunization of Balb/c mice with three PCT fragments - N-terminal fragment of PCT, calcitonin and katacalcin. For PCT immunodetection antibodies specific to different fragments of the molecule should be used. Calibration curves of sandwich immunoassays for PCT detection in blood, utilizing MAbs with different epitope specificity, are shown on Fig. 38.

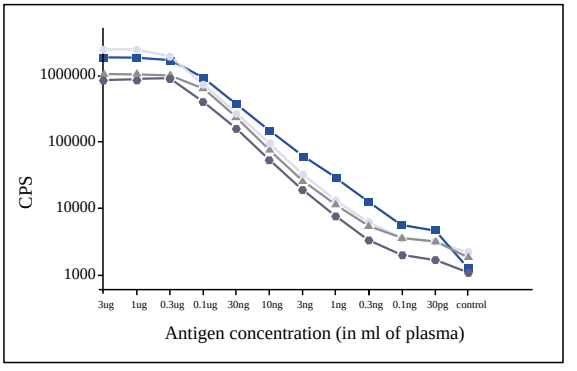


Fig. 38. Calibration curves of several PCT sandwich immunoassays.
Capture MAbs: 1µg/well
Antigen: human recombinant PCT
Detection Eu-labeled MAbs: 0.1 µg/well
- *16B5 (central part) – 42 (N-terminal fragment)*
- *24B2 (central part) – 13B9 (central part)*
- *14C12 (C-terminal part) – 14A2 (central part)*
- *27A3 (N-terminal fragment) – 22A11 (C-terminal part)*

The recommended pairs for sandwich immunoassay:

16B5 ——— 42
14A2 ——— 42
24B2 ——— 38F11
13G11 ——— 42
16B5 ——— 44D9
24B2 ——— 13B9
14C12 ——— 38F11

The antibody pair 24B2 – 13B9 can be used for the immunodetection of both PCT and free calcitonin.

MAbs may also be used for PCT detection in immunoblotting. All MAbs recognize PCT in Western Blotting after SDS-electrophoresis in reducing conditions (Fig 39).

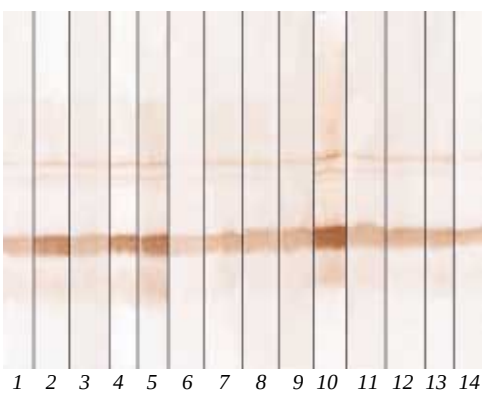


Fig. 39. Immunodetection of human recombinant PCT in Western blotting by monoclonal antibodies specific to different PCT fragments after 15 % SDS-PAGE in reducing conditions.
Antigen: 100 ng/track.

1 – MAb 6F10
2 – MAb 27A3
3 – MAb 38F11
4 – MAb 44D9
5 – MAb 42
6 – MAb 13B9
7 – MAb 13F2
8 – MAb 13G11
9 – MAb 14A2
10 – MAb 16B5
11 – MAb 24B2
12 – MAb 14C12
13 – MAb 18B7
14 – MAb 22A11

Ordering information

Clone	Cat. #	Specificity (part of PCT)	Subclass	Application
6F10	4PC47	N-terminal fragment	IgG1	EIA, Sandwich immunoassay (capture), WB
27A3	4PC47	N-terminal fragment	IgG2a	EIA, Sandwich immunoassay (capture), WB
38F11	4PC47	N-terminal fragment	IgG1	EIA, Sandwich immunoassay (capture), WB
44D9	4PC47	N-terminal fragment	IgG2a	EIA, Sandwich immunoassay (detection), WB
42	4PC47	N-terminal fragment	IgG2a	EIA, Sandwich immunoassay (detection), WB
13B9	4PC47	calcitonin (central part)	IgG2a	EIA, Sandwich immunoassay (detection), WB
13F2	4PC47	calcitonin (central part)	IgG1	EIA, Sandwich immunoassay (capture), WB
13G11	4PC47	calcitonin (central part)	IgG1	EIA, Sandwich immunoassay (capture), WB
14A2	4PC47	calcitonin (central part)	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
16B5	4PC47	calcitonin (central part)	IgG2b	EIA, Sandwich immunoassay (capture, detection), WB
24B2	4PC47	calcitonin (central part)	IgG1	EIA, Sandwich immunoassay (capture), WB
14C12	4PC47	katacalcin (C-terminal part)	IgG1	EIA, Sandwich immunoassay (capture), WB
18B7	4PC47	katacalcin (C-terminal part)	IgG1	EIA, Sandwich immunoassay (detection), WB
22A11	4PC47	katacalcin (C-terminal part)	IgG1	EIA, Sandwich immunoassay (detection), WB

References:
1. Le Moullec J.M., et al. The complete sequence of human procalcitonin. // FEBS Lett., 167 (1), 93-97 (1984)
2. Assicot M, et al. High serum procalcitonin concentrations in patients with sepsis and infection. // Lancet, 341, 515-18 (1993)
3. Meisner M., Reinhart K. Is procalcitonin really a marker of sepsis? // Int. J. Intensive Care, 8 (1), 15-25. (2001)
4. Potapov EV., et al. Elevated donor cardiac troponin T and procalcitonin indicate two independent mechanisms of early graft failure after heart transplantation. // Int. J. Cardiol., 92 (2-3), 163-7 (2003)

XVIII Brain S-100 Protein

1. Human Brain 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*

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 synthesized by astroglial cells and is mainly presented by two isoforms, alpha-beta heterodimer (S-100a) and 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 levels of S-100 correlate 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. 40).

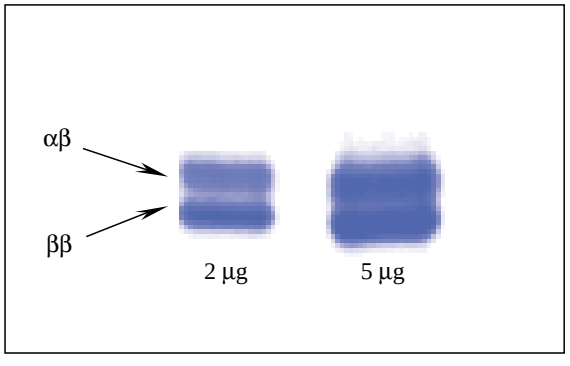


Fig. 40. Native gel electrophoresis of S-100 protein (by Ornstein – Davis)
Antigen loaded: *lane 1 – 2 µg*
lane 2 –5 µg
Gel staining: *Coomassie brilliant blue R-250*

Ordering information

Product	Cat. #	Purity	Source
Human S-100	8S9h	> 95 %	Human brain
Human S-100, beta-beta homodimer	8S9-2h	> 95 %	Human brain

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*

Hybridomas producing anti-S-100 protein antibodies were generated after immunization of Balb/c mice with purified human brain S-100 protein. The best combinations of MAbs for sandwich immunoassay:

8B10 —— 6G1 (Fig. 41)
3B10 —— 6G1

The Mabs are working also in Western Blotting (Fig. 42).

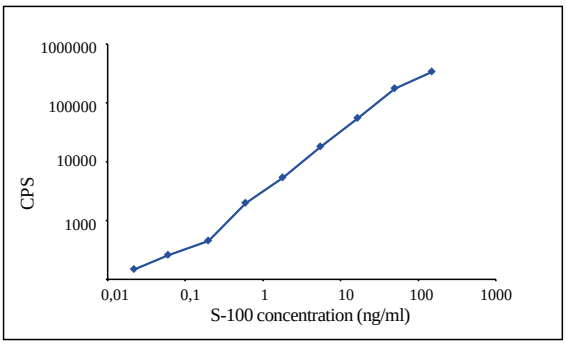


Fig. 41. S-100 calibration curve.
One step assay in streptavidin coated plates.
- capture antibodies: *biotinylated 8B10; 200 ng/well*
- detection antibodies: *Eu-labeled 6G1; 200 ng/well*
Antigen: *S-100 protein from human brain*
Incubation time: *20 min*
Temperature: *20 °C*

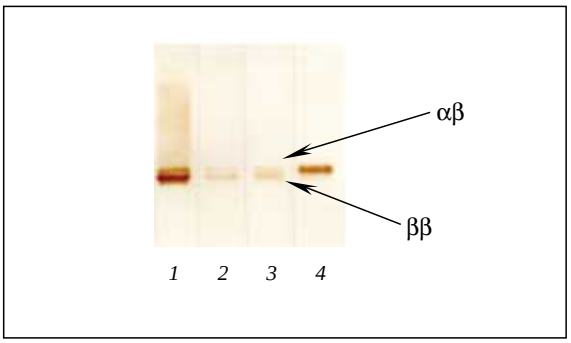


Fig. 42. Interaction of monoclonal antibodies with S100 protein from human brain in Western blotting (after native gel electrophoresis by Ornstein – Davis)

Antigen loaded: 1µg
1 - MAb 4B3
2 - MAb 8B10
3 - MAb 6G1
4 - MAb 3B10.

Ordering information

Clone	Cat. #	Specificity in WB	Subclass	Application
3B10	4S37	αβ	IgG2a	EIA, Sandwich immunoassay (capture), WB
4B2	4S37	αβ and ββ	IgG2a	WB
8B10	4S37	αβ and ββ	IgG1	EIA, Sandwich immunoassay (capture), WB
6G1	4S37	αβ and ββ	IgG1	EIA, Sandwich immunoassay (detection), WB