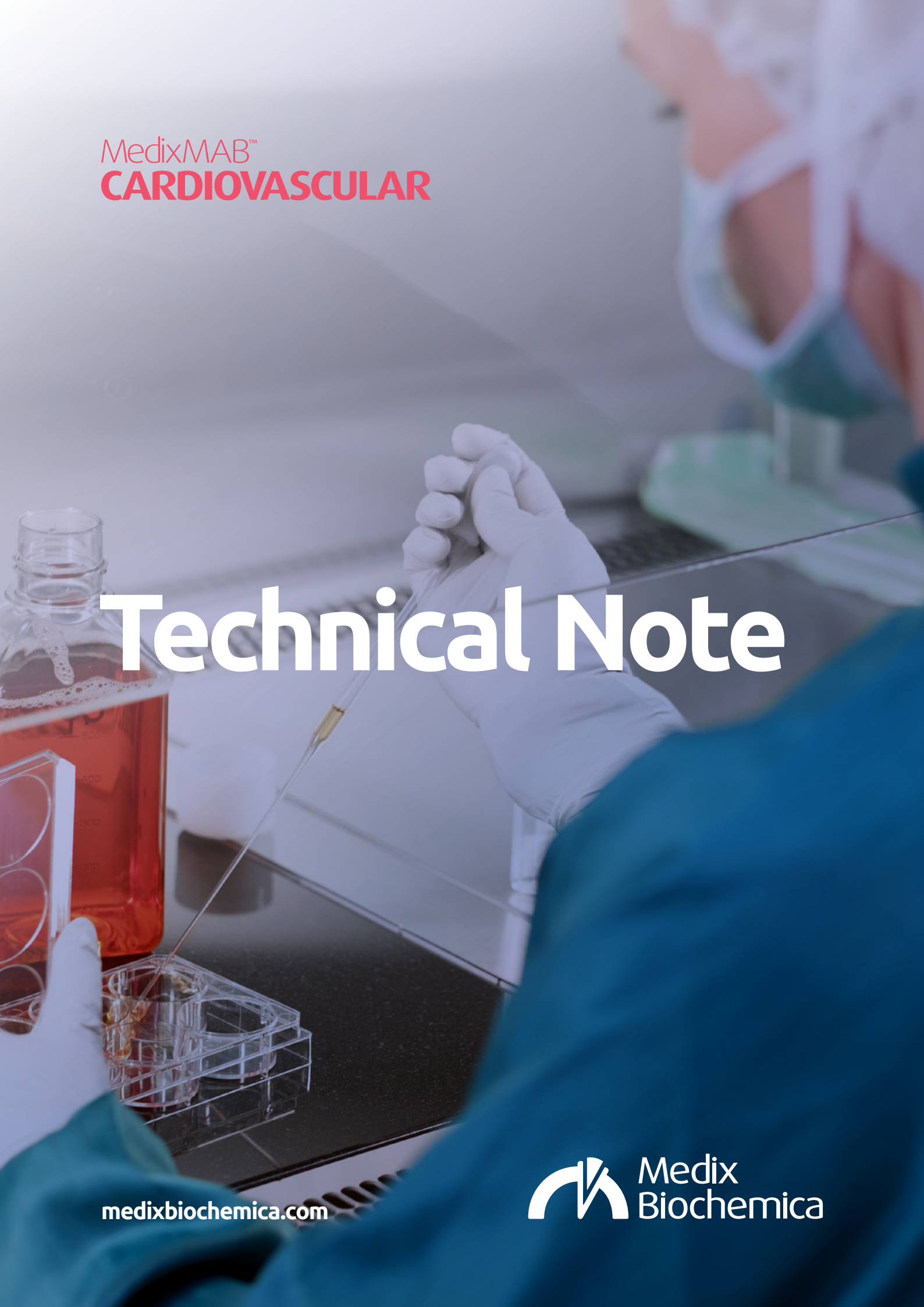
MedixMAB™
CARDIOVASCULAR

Technical Note



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Introduction

Cardiovascular diseases (CVD) are a group of disorders affecting the heart and blood vessels. Accounting for an estimated one third of all deaths worldwide, CVDs are the number one global cause of mortality. Rapid diagnostics as well as reliable identification and monitoring of high-risk individuals is vital for lowering risk of CVD-related death and disability. Cardiac biomarkers are a varied group of molecules whose increased concentrations relate to the presence and the risk of future cardiovascular events. There is a growing need for accurate biomarkers for diagnostics and risk stratification, as efficient treatment and prevention of CVD rely on early detection of patients with, or at risk of, these diseases.

Several cardiac markers are currently used in clinical practice to support diagnosis, risk assessment, and treatment follow-up related to different CVD pathophysiological processes. For example, cardiac muscle damage causes cardiac troponin (cTn) to be released into the bloodstream by myocardial cells. Elevated serum levels of cTn thus indicate heart injury, and immunoassays for cTn form the cornerstone of diagnosing acute myocardial infarction (AMI). Current cTn assay methods allow AMI to be ruled out as early as one hour after onset of symptoms. As another example, the blood concentration of commonly used cardiac biomarker D-dimer correlates with the thrombolytic activity of the body. D-dimer immunoassays thus provide a useful tool for excluding pulmonary embolism and deep vein thrombosis.

Medix Biochemica provides a wide selection of premium-quality monoclonal antibodies for cardiac marker detection. Their optimized, industrial-scale in vitro production methods, certified batch-to-batch consistency, and expert customer service have made Medix Biochemica one of the most important antibody suppliers for the *in vitro* diagnostic (IVD) community.

cTnI & cTnT

Troponin is a regulatory protein complex involved in the calcium-mediated process of muscle contraction and relaxation in cardiac and skeletal muscle filaments. The troponin complex consists of three subunits: troponin I, T, and C. The names of the subunits reflect their biological functions; troponin I affects the myosin-actin interactions, troponin T mediates the binding of troponin to tropomyosin, and troponin C binds calcium ions.[1]

Troponin I and T have myocardial tissue-specific isoforms, cardiac troponin I (cTnI) and cardiac troponin T (cTnT). cTnI is expressed exclusively in the heart, but cTnT has occasionally also been detected in diseased skeletal muscles. The same isoform of troponin C is expressed in both myocardium and slow-twitch skeletal muscles and therefore it cannot be used as a cardiac marker, whereas cTnI and cTnT have become the most widely used biomarkers for detecting myocardial injury. After cardiac muscle damage, cardiac troponin is released into the bloodstream by myocardial cells due to loss of membrane integrity. Therefore, elevated serum levels of cTnI and cTnT indicate heart injury, but are independent of the mechanism causing it.[1,2]

Serum cTnI and cTnT have superior specificity and sensitivity compared to other routinely used biomarkers, such as creatine kinase (CK-MB), lactate dehydrogenase, and myoglobin. Therefore, measurement of cardiac troponin is the cornerstone of diagnosis of acute myocardial infarction (AMI). Current guidelines recommend serial measurements of cardiac troponin levels and the use of the 99th percentile as an assay-specific cardiac troponin level cut-off value for AMI diagnosis. Similar to CK-MB, cardiac troponin is typically detectable within 4–6 hours after the onset of symptoms. However, current assay technologies allow fast and precise detection of cardiac troponin at very low concentrations, and enable ruling out AMI as early as an hour after symptom onset. After infarction, cardiac troponin concentrations remain elevated for several days.[3,4]

In addition to AMI, elevated levels of cTnI and cTnT may occur due to other conditions, such as renal failure, sepsis, and hypertension. Furthermore, high cTnI and cTnT concentrations serve as an adverse prognostic indicator in patients with acute coronary syndromes (ACS).[2]

cTnI

The 210 residues long amino acid sequence of the 24-kDa human cTnI is highly conserved between various animals, which enables several human cTnI assays to be effectively adapted for animal use. The initial section of the protein sequence, however, is less conserved, and thus human-specific antibodies are also available.[1]

Medix Biochemica has more than three decades of experience in producing high-quality monoclonal antibodies against cTnI. Currently, the MedixMAB product portfolio includes seven anti-cTnI monoclonal antibodies (9701, 9703, 9705, 9707, 9708, 9709 and RC9750). In addition, Medix Biochemica offers a recombinant cTnI antigen.

Anti-human cTnI monoclonal antibodies and recombinant antigen

cTnI antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
9701	100129	5	36	IgG_1	ELISA, LF
9703	100181	5	12	IgG_1	ELISA, LF
9705	100125	1	36	IgG_1	ELISA, LF
9707	100180	5	18	IgG_1	ELISA, LF
9708	100523	5	N/D	IgG_1	ELISA
9709	100524	5	N/D	IgG_1	ELISA
RC9750	700050	5	N/D	IgG_1	ELISA

cTnI antigen	Product code
Recombinant cTnI, 100 µg	610102

Pair recommendations

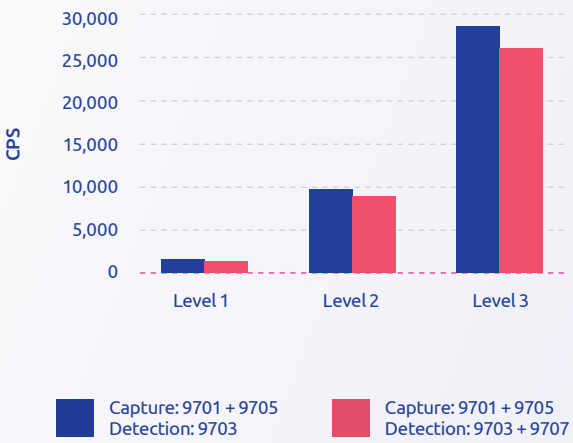
Capture \ Detection	9701	9703	9705	9707	9708	9709
9701	–	+	+	+	+	+
9703	–	–	+	–	+	+
9705	+	+	–	+	–	–
9707*	–	–	–	–	–	–
9708	–	–	–	–	–	–
9709	–	+	–	–	–	–

Capture mAb recommendations for antigens

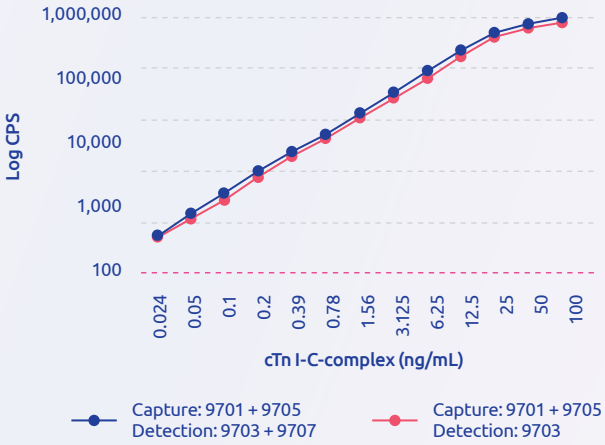
Capture \ Antigen	cTnI	cTn I-C	cTn I-T-C
9701	+	+	–
9703	+	+	–
9705	–	+	+
9707*	–	(+)	(+)
9708	–	–	–
9709	–	+	+

* 9707 is not recommended as a capture antibody due to the cross-reactivity with skeletal Troponin I (14%)

cTnI detection



cTnI standard curve



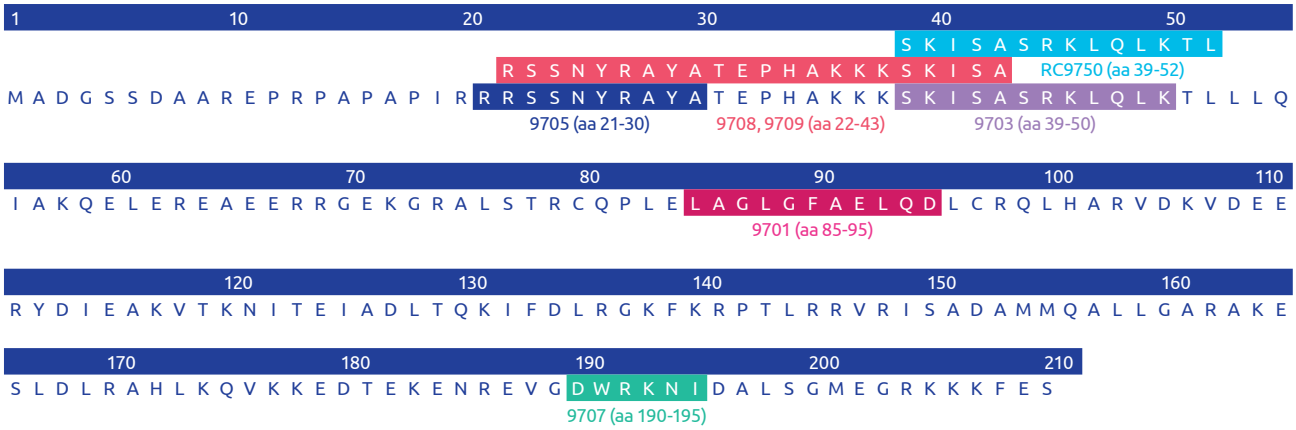
For specific and sensitive determination of cardiac troponin I, 2+1 or 2+2 antibody approach is recommended to be used. Capture: 9701 + 9705, detection: 9703 or 9703 + 9707.

Kinetic parameters

cTnI antibody	Association rate constant, k_{on}	Dissociation rate constant, k_{off}	Affinity constant, K_A
9701	4.1×10^5 1/Ms	5.6×10^{-4} 1/s	$K_A = 0.7 \times 10^9$ 1/M, $K_D = 1.4 \times 10^{-9}$ M = 1.4 nM
9703*	N/D	N/D	$K_A = 1.0 \times 10^9$ 1/M, $K_D = 1.0 \times 10^{-9}$ M = 1.0 nM
9705	7.0×10^5 1/Ms	4.5×10^{-5} 1/s	$K_A = 1.6 \times 10^{10}$ 1/M, $K_D = 6.4 \times 10^{-11}$ M = 0.064 nM
9707	1.9×10^6 1/Ms	5.8×10^{-6} 1/s	$K_A = 3.3 \times 10^{11}$ 1/M, $K_D = 3.0 \times 10^{-12}$ = 0.003 nM
9708	2.8×10^5 1/Ms	1.7×10^{-5} 1/s	$K_A = 1.6 \times 10^{10}$ 1/M, $K_D = 5.9 \times 10^{-11}$ M = 0.059 nM
9709	2.4×10^5 1/Ms	3.5×10^{-5} 1/s	$K_A = 6.9 \times 10^9$ 1/M, $K_D = 1.4 \times 10^{-10}$ = 0.14 nM

* Affinity constant for 9703 has been determined using cTnI antigen, and for other antibodies cTn I-T-C antigen.

Binding epitopes

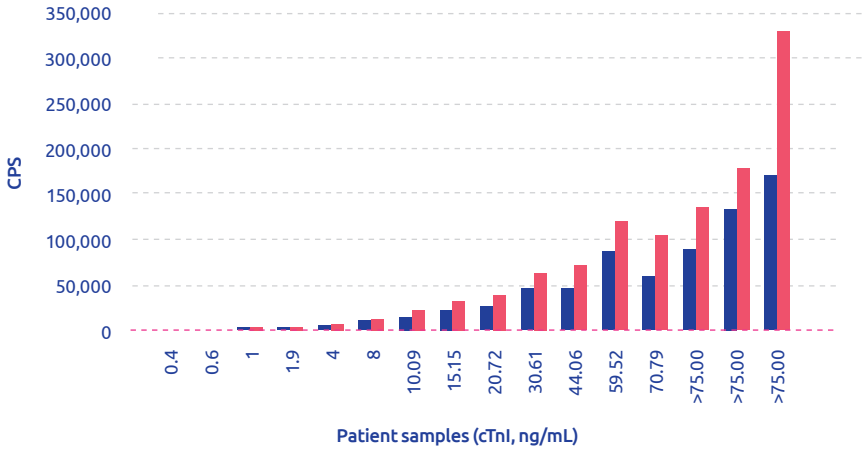


Product	Epitope (amino acids)
9701	85–95
9703	39–50

Product	Epitope (amino acids)
9705	21–30
9707	190–195

Product	Epitope (amino acids)
9708	22–43
9709	22–43
RC9750	39–52

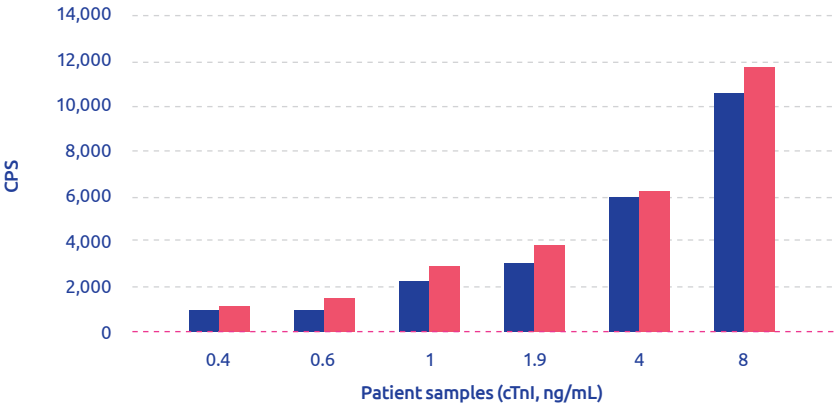
Results with patient samples



Patient samples with elevated cTnI levels were measured with in-house FIA. The cTnI concentrations had been determined earlier using Beckman Coulter DxI 800 Immunoassay System.

Capture: 9701 + 9705
Detection: 9703

Capture: 9701 + 9705
Detection: 9703 + 9707



A zoom-in figure of the low-level cTnI patient samples. Both assay set-ups can differentiate the low level samples, with assay format 2 + 2 giving slightly better resolution. Standard FIA conditions were not specifically optimized for high sensitivity cTnI measurements.

Capture: 9701 + 9705
Detection: 9703

Capture: 9701 + 9705
Detection: 9703 + 9707

cTnT

The 288 amino acids long, 34.6 kDa human cardiac troponin T (cTnT, isoform 6) protein binds to tropomyosin and attaches the troponin complex to the thin filament within cardiomyocytes. Cardiac tissue expresses several distinct cTnT isoforms as a result of alternative exon splicing.[5]

The MedixMAB product portfolio includes seven monoclonal antibodies for the detection of cTnT (3701, 3703, 3708, 3709, 3710, 3711 and 3712). In addition, Medix Biochemica offers a recombinant cTnT antigen.

Anti-human cTnT monoclonal antibodies and recombinant antigen

cTnT antibody	Product code	Concentration (mg/mL)	Subclass	Applications tested
3701	100660	5	IgG_1	ELISA
3703	100700	5	IgG_1	ELISA
3708	100704	5	IgG_1	ELISA
3709	100705	5	IgG_{2b}	ELISA, CLIA
3710	100698	5	IgG_1	ELISA
3711	100701	5	IgG_1	ELISA
3712	100699	5	IgG_1	ELISA, CLIA

cTnT antigen	Product code
Recombinant cTnT, 100 µg	610101

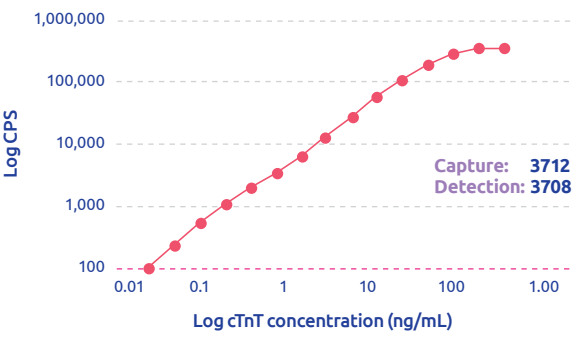
Pair recommendations

Capture \ Detection	3701	3703	3708	3709	3710	3711	3712
3701	–	–	–	–	–	–	–
3703	–	–	–	–	+	+	+
3708	+	+	–	–	+	+	+
3709	+	+	–	–	+	+	+
3710	–	+	+	+	–	–	–
3711	–	+	–	–	–	–	–
3712	–	+	+	+	–	–	–

Binding epitopes

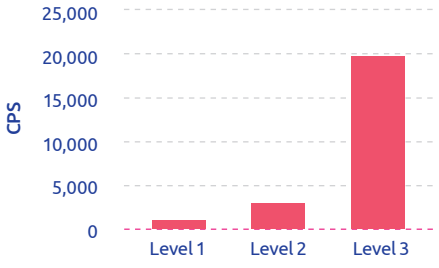
Clone	Epitope (amino acids)
3701	N/D
3703	N/D
3708	121–133 (VSLKDRIERRRAE)
3709	121–133 (VSLKDRIERRRAE)
3710	139–152 (RIRNEREKERQNRL)
3711	139–152 (RIRNEREKERQNRL)
3712	139–152 (RIRNEREKERQNRL)

cTnT standard curve



cTnT detection

Cardiac troponin T in Liquichek™ Cardiac Markers Plus Controls (Bio-Rad, Cat 180X, TriLevel).



Capture: **3712** Detection: **3708**

D-dimer

D-dimers are fragments of fibrin that form when blood clots are broken down in an enzymatic process. Plasmin cleaves cross-linked insoluble fibrin molecules into differently sized fibrin degradation products (FDPs). D-dimers are among these FDPs, and consist of two cross-linked D fragments of the fibrin protein. Under physiological conditions, D-dimers are usually non-covalently bonded to E-fragments, which are also fibrin fragments.[6–11]

In healthy individuals, the plasma concentration of D-dimers is low. However, many pathological conditions increase the thrombolytic activity of the body and thus correlate with an increased D-dimer blood concentration. Such conditions include thrombosis, malignancies, infections, and severe inflammation, for example. The physiological plasma levels of D-dimer are higher in women than in men, and the D-dimer concentration also increases with age.[8,9]

In clinical diagnostics, D-dimer testing can be utilized for ruling out pulmonary embolism and deep vein thrombosis (DVT). D-dimer is detectable in blood approximately two hours after signs of thrombus formation and has a half-life of eight hours. Although D-dimer is not specific for thromboembolic diseases, it is used to support the diagnosis of disseminated intravascular coagulation (DIC), as well as in monitoring patients during and after anticoagulant treatment for recurrent DVT.[7,8,11,12]

Medix Biochemica's product selection includes eight high-quality monoclonal IgG antibodies with versatile specificities for the detection of D-dimer. MedixMAB D-dimer antibodies have varying specificities towards fibrinogen and fibrin degradation products (FDPs). This allows their utilization in the specific detection of the D-dimer monomer (~180 kDa) as well as in different sandwich ELISA assays. The pairing properties have proven suitable for sandwich ELISA applications.

Anti-human D-dimer monoclonal antibodies

D-dimer antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
1401	100204	5	18	IgG_3	ELISA, IT
1402	100205	5	12	IgG_{2b}	ELISA, IT
1403	100228	5	36	IgG_{2a}	ELISA, IT
1404	100479	5	24	IgG_1	ELISA, IT
1405	100480	5	36	IgG_1	ELISA, IT
1407	100482	5	24	IgG_1	ELISA, IT
1408	100799	5	N/D	IgG_1	ELISA
1409	100800	5	N/D	IgG_1	ELISA

Pair recommendations

Capture \ Detection	1401	1402	1403	1404	1405	1407	1408	1409
1401	–	–	+	+	+	+	++	++
1402	–	–	–	+	–	–	–	–
1403	–	–	–	+	–	+	–	–
1404	–	+	+	–	+	+	–	–
1405	+	+	+	+	–	+	+	–
1407	+	+	–	–	–	–	–	–
1408	++	+	+	–	–	+	–	++
1409	+	–	–	–	–	–	++	–

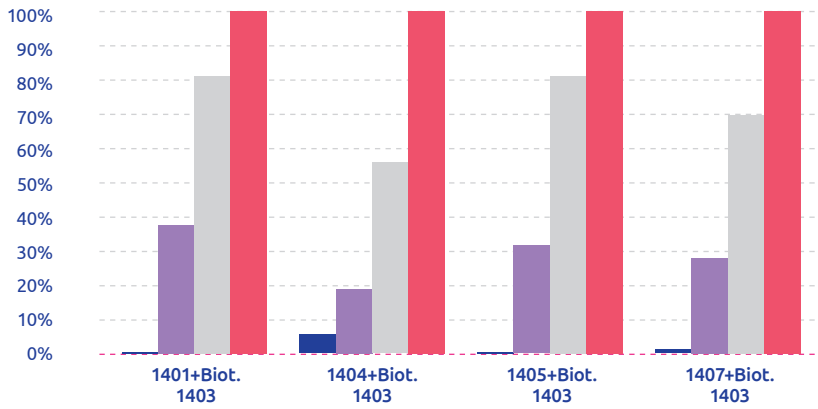
Binding properties

D-dimer antibody	FDP-E	FDP-D	FDP-X	FDP-Y	Fibrinogen
1401	–	–	–	+	–
1402	–	+	+	+	+
1403	–	+	+	+	+
1404	–	+	–	–	–
1405	–	+	–	–	–
1407	–	+	+	+	–
1408	–	+	–	+	–
1409	–	+	+	+	–

D-dimer antibodies bind to fibrin degradation products (FDP-D, FDP-X, and FDP-Y) and fibrinogen with differing specificities. The molecular configuration of the fragments has been described in Walker & Nesheim, 1999 .[11]

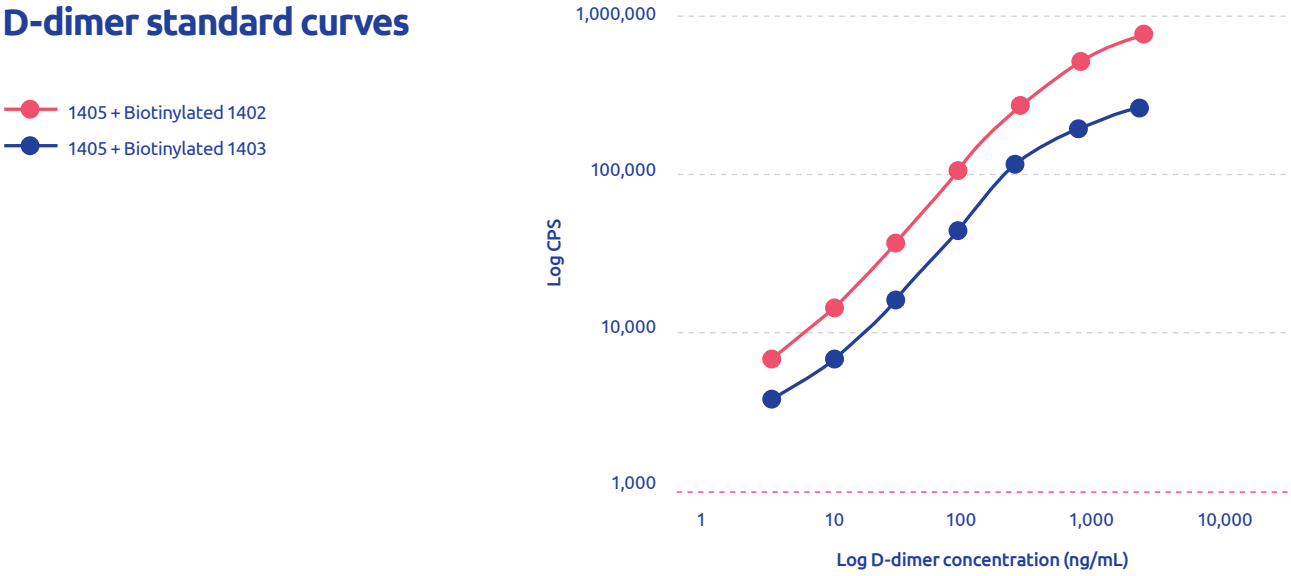
Results with serum controls

- Buffer
- BioRad D-Dimer Liquichek™ Level 1=550 ng/mL
- BioRad D-Dimer Liquichek™ Level 2=1600 ng/mL
- BioRad D-Dimer Liquichek™ Level 3= 3200 ng/mL



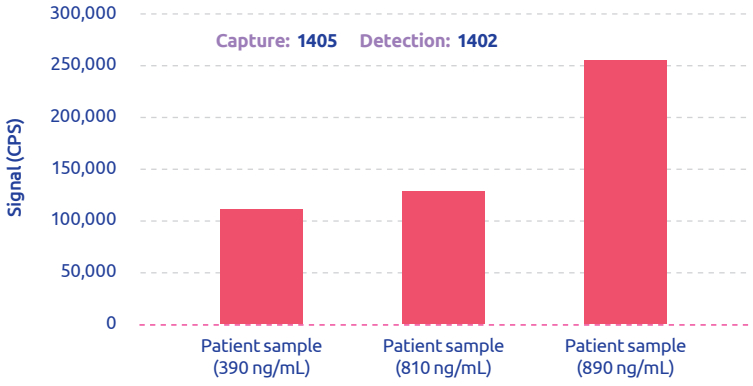
D-dimer monoclonal antibodies were tested in sandwich FIA using Bio-Rad Liquichek™ D-Dimer Quality Control materials. The 100% level equals to 3200 D-dimer ng/ml (Fibrinogen Equivalent Units; FEU). The combination of clones 1405 (capture) and 1403 (label; biotinylated, detection using Eu-labeled streptavidin) resulted in the least amount of non-specific binding combined with high detection sensitivity.

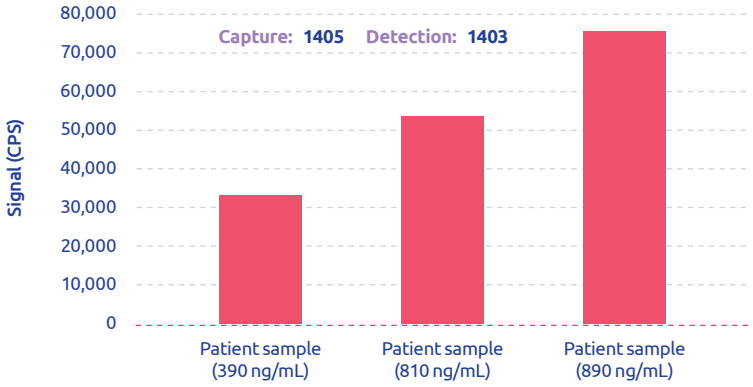
D-dimer standard curves



D-dimer detection in patient samples

Patient samples with variable concentrations of D-dimer were analyzed using 1405, 1402 and 1403. Standard FIA conditions, that were not specifically optimized for any of the antibodies, were applied. This may increase variance in the results in samples containing low concentrations of the D-dimer. D-dimer concentrations had been measured earlier by a Siemens Innovance® D-dimer test.





NT-proBNP & proBNP

Natriuretic peptides function as protective hormones that counteract the physiological abnormalities of heart injury and myocardial dysfunction through their diuretic, natriuretic, and vasodilatory effects. The most relevant biomarkers of this family include B-type natriuretic peptide (BNP) and N-terminal prohormone of natriuretic peptide (NT-proBNP), which have been established as effective novel biomarkers for heart failure.[13,14]

The cardiac hormone BNP is predominantly released from cardiac myocytes in ventricles in response to myocardial wall stress secondary to volume and pressure overload. Initially, BNP is secreted as a biologically inactive 108-amino acid pro-BNP that is proteolytically cleaved to form the 32-amino acid bioactive BNP and its biologically inactive N-terminal fragment, the 76-amino acid NT-proBNP molecule.[13] NT-proBNP and BNP are secreted in a 1:1 ratio, but due to the longer half-life of NT-proBNP in the circulation (90–120 minutes compared with 20 minutes for BNP) the plasma concentrations of NT-proBNP are usually 6–10 times higher than BNP.[14–16]

The levels of both BNP and NT-proBNP are significantly increased in the plasma of patients with asymptomatic and symptomatic cardiac dysfunction and provide a hemodynamic measure of myocardial injuries.[16] Therefore, serum BNP and NT-proBNP tests have become valuable tools to confirm or exclude the presence of cardiovascular diseases, heart failure in particular. In addition, BNP and NT-proBNP assays have shown good clinical and statistical performance in providing independent prognostic information for risk stratification.[17–19]

Due to its longer half-life, NT-proBNP has the advantage of being more stable than BNP in clinical testing. However, NT-proBNP is affected more by age and renal function than BNP, and therefore requires careful assessment in the elderly and in patients with compromised renal function.[15]

Medix Biochemica offers seven high-quality monoclonal antibodies for the detection of NT-proBNP (1306, 1307, 1308, 1309, 1310, 1311, and 1312). In addition, the MedixMAB product portfolio includes one recombinant NT-proBNP antigen and one recombinant proBNP antigen in three product sizes.

Anti-human NT-proBNP monoclonal antibodies and recombinant antigens

NT-proBNP antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
1306	100521	5	18	IgG_1	ELISA, CLIA, LF
1307	100719	5	12	IgG_1	ELISA, CLIA, LF
1308	100712	5	24	IgG_{2b}	ELISA, CLIA, LF
1309	100710	5	12	IgG_1	ELISA, CLIA, LF
1310	100718	5	N/D	IgG_1	ELISA, CLIA, LF
1311	100716	5	N/D	IgG_1	ELISA, CLIA, LF
1312	100717	5	N/D	IgG_1	ELISA, CLIA, LF

NT-proBNP & proBNP antigen	Product code
Recombinant NT-proBNP, 100 µg	610090
Recombinant proBNP, 50 µg	710017
Recombinant proBNP, 500 µg	710043
Recombinant proBNP, 1000 µg	710042

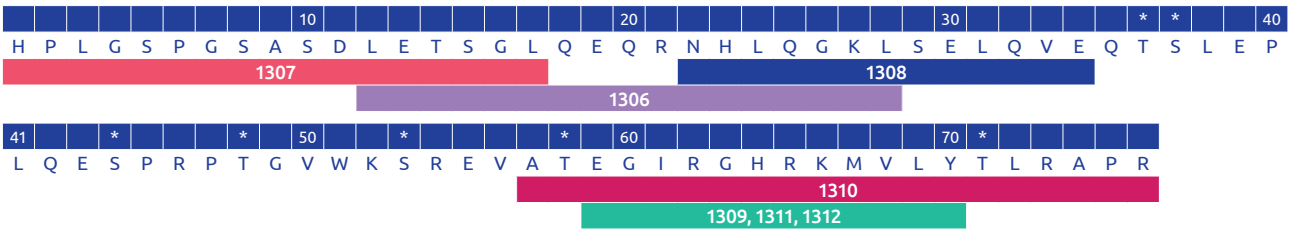
Pair recommendations

Capture \ Detection	1306	1307	1308	1309	1310	1311	1312
1306	–	+	–	+	+	+	+
1307	–	–	+	+	+	+	+
1308	–	+	–	+	+	+	+
1309	+	+	+	–	–	–	–
1310	+	+	+	–	–	–	–
1311	+	+	+	–	–	–	–
1312	+	+	+	–	–	–	–

Kinetic parameters

NT-proBNP antibody	Association rate constant, k_{on}	Dissociation rate constant, k_{off}	Dissociation constant, K_D
1306	7.45×10^{5} 1/Ms	1.70×10^{-4} 1/s	$K_D = 2.3 \times 10^{-10}$ M

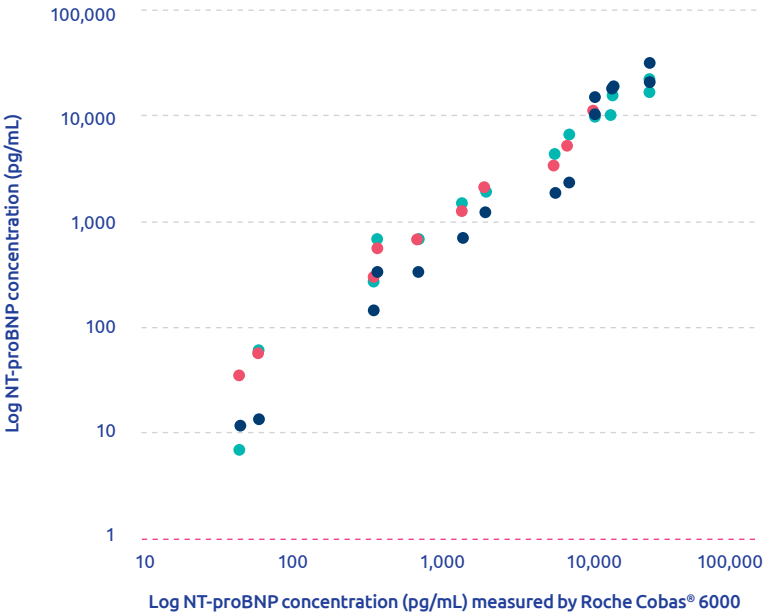
Binding epitopes



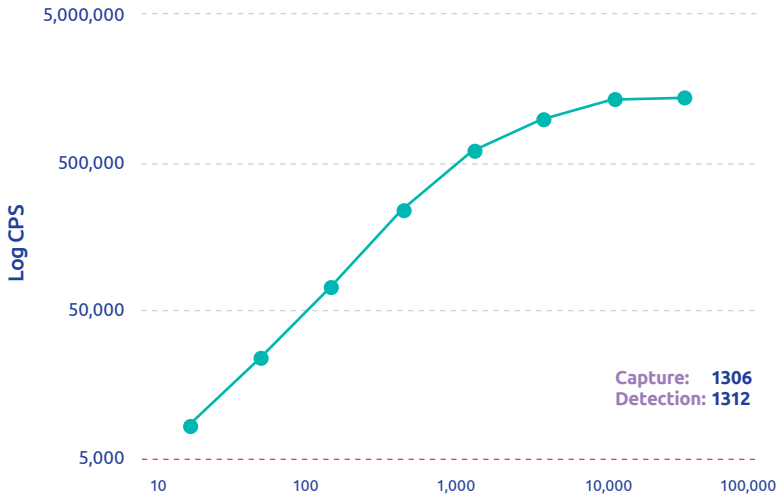
* Potential glycosylation sites

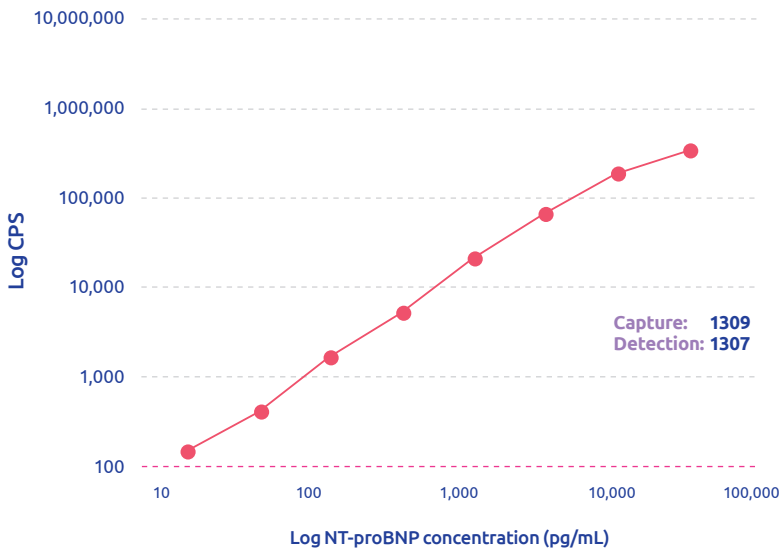
Correlation to reference IVD assay

- Capture: 1306 Detection: 1309
- Capture: 1306 Detection: 1312
- Capture: 1309 Detection: 1307



NT-proBNP standard curves





ST2

ST2 belongs to the interleukin (IL)-1 receptor-like family of proteins expressed in cardiomyocytes in response to mechanical stress. The protein is expressed both in a transmembrane receptor form (ST2L) and a soluble decoy receptor form (sST2). IL-33, which is involved in reducing fibrosis and hypertrophy, is the ligand for both forms. Binding of IL-33 to ST2L promotes signaling that exerts protective effects on cardiomyocytes. On the contrary, sST2 acts as a decoy receptor; when it is bound to IL-33 it prevents the beneficial signaling thus inducing fibrosis and hypertrophy.[20,21]

Current clinical assays measure sST2 whose elevated concentrations are strongly associated with adverse outcomes in heart failure.[39] The recommended cut-off for sST2 concentration in heart failure is 35 ng/mL, when assessed by Presage ST2 assay which has been approved for prognostication of heart failure in Europe and USA.[40] Unlike natriuretic peptides, sST2 is not affected by age, sex, body mass index, and renal function when used as biomarkers in a clinical setting.[39] Furthermore, sST2 is the strongest predictor of mortality from both acute and chronic heart failure.[26,41,42] Besides being a prognostic biomarker for mortality, sST2 could be used to guide treatment decisions in the future. It has been demonstrated that patients with elevated sST2 levels may particularly benefit from high-dose beta blockers and mineralocorticoid inhibitors.[22–28]

Medix Biochemica offers seven monoclonal antibodies for the detection of ST2. Three of the ST2 antibodies recognize both free sST2 and sST2 bound to interleukin 33 (IL-33) (10201, 10202 and 10203), while the other four are specific to free sST2 alone (10204, 10205, 10206 and 10207). In addition, the MedixMAB product portfolio includes one recombinant ST2 antigen.

Anti-human ST2 monoclonal antibodies and recombinant antigen

ST2 antibody	Product code	Concentration (mg/mL)	Specificity	Shelf life (months at +2–8°C)	Subclass	Applications tested
10201	100680	5	Free sST2 and sST2 bound to IL-33	N/D	IgG_1	ELISA
10202	100681	5	Free sST2 and sST2 bound to IL-33	N/D	IgG_1	ELISA
10203	100682	5	Free sST2 and sST2 bound to IL-33	12	IgG_1	ELISA
10204	100683	5	Free sST2	N/D	IgG_1	ELISA
10205	100684	5	Free sST2	N/D	IgG_1	ELISA
10206	100685	5	Free sST2	12	IgG_1	ELISA
10207	100686	5	Free sST2	N/D	IgG_1	ELISA

ST2 antigen	Product code
Recombinant ST2, 50 µg	710020
Recombinant ST2, 500 µg	710047
Recombinant ST2, 1000 µg	710046

Pair recommendations

Capture \ Detection	10201	10202	10203	10204	10205	10206	10207
10201	–	–	–	+	+	+	+
10202	–	–	–	+	+	+	+
10203	–	–	–	+	+	+	+
10204	+	+	+	-	+	–	–
10205	+	+	+	+	–	–	–
10206	+	+	+	–	–	–	–
10207	+	+	+	–	–	–	–

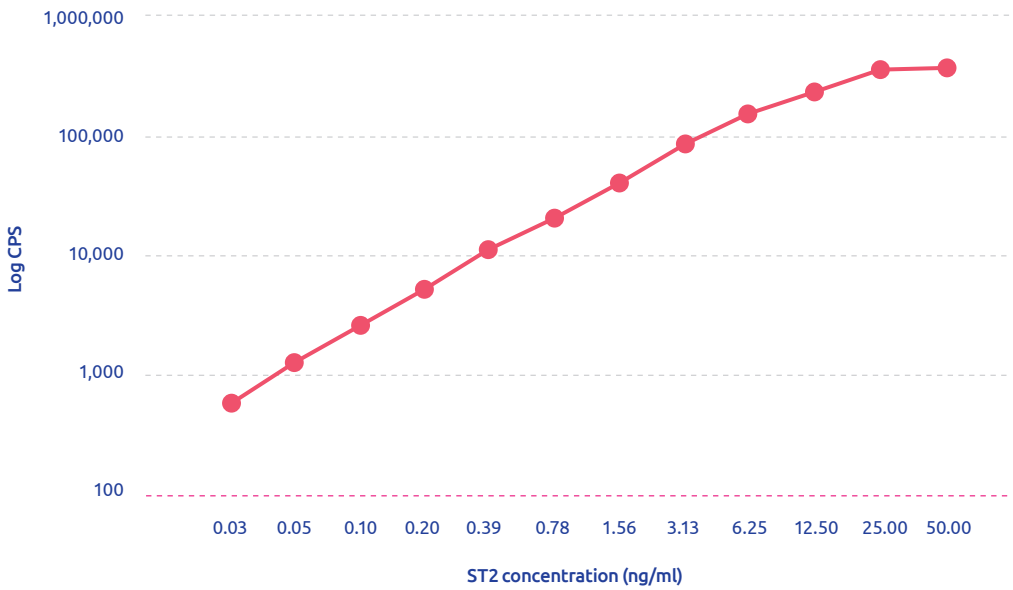
ST2 detection

Presage ST2 Controls
(Critical Diagnostics, Cat BC-1066E)

Capture: **10201**
Detection: **10206**

ST2 standard curve

Capture: **10201**
Detection: **10206**



Galectin-3

Galectin-3 (Mac-2 antigen, IgE-binding protein, L-29, or CBP30) is a soluble 35-kDa lectin that binds to the β-galactoside sugars that are found on several proteins.[29] It consists of two characteristic domains: a C-terminal carbohydrate recognition domain (CRD), and an N-terminal domain with a unique proline-glycine-alanine-thyrosine-rich (PGAY) repeat motif that enables oligomerization.[30,31] Galectin-3 is abundantly expressed across various different cell and tissue types. It is found both intracellularly and extracellularly,[31] and its biological functions are dependent on the subcellular localization. Galectin-3 binds to several different proteins and mediates diverse physiological responses, including cell cycle, cell adhesion and apoptosis, tissue development, immune responses, neoplastic transformation, angiogenesis and metastasis.[30–32]

Galectin-3 is a pro-inflammatory and pro-fibriotic marker involved in fibrosis of various organs, including heart, vessels, lungs, liver, and kidneys.[29,33] Its expression is upregulated in chronic inflammatory diseases, heart failure, hypertension and atherosclerotic lesions, and it is involved in several pathophysiological processes including cancer, liver cirrhosis, and diabetes mellitus.[29,34,35] In addition, galectin-3 induces pathologic atrial remodeling in atrial fibrillation patients.[36–38] It is an accurate diagnostic and prognostic marker of poor outcomes and high mortality in patients with myocardial ischemia, acute ischemic stroke and chronic heart failure, whose prognosis is dependent on accurate and timely diagnosis.[38–40]

Medix Biochemica offers five monoclonal antibodies for the detection of galectin-3, and a recombinant antigen.

Anti-human Galectin-3 monoclonal antibodies and recombinant antigen

Galectin-3 antibody	Product code	Concentration (mg/mL)	Subclass	Applications tested
10301	100730	5	IgG_1	ELISA
10302	100731	5	IgG_1	ELISA
10303	100732	5	IgG_1	ELISA
10304	100733	5	IgG_1	ELISA
10305	100734	5	IgG_1	ELISA

Galectin-3 antigen	Product code
Recombinant Galectin-3, 100 µg	610144

Pair recommendations

Capture \ Detection	10301	10302	10303	10304	10305
10301	–	+	+	+	+
10302	+	–	–	+	+
10303	+	–	–	+	+
10304	+	+	+	–	–
10305	+	+	+	–	–

FABP3

Fatty acid-binding proteins (FABP) are a family of lipid chaperones involved in the transport and metabolism of fatty acids. So far, at least nine members of the family have been identified. FABPs are 14–15 kDa proteins that reversibly bind hydrophobic ligands, such as fatty acids, with high affinity.[19] Heart-type FABP (H-FABP, also known as FABP3) is a cytosolic, low-molecular-weight protein present in abundance in the myocardium, but also in small quantities in the brain, kidney and skeletal muscle.[13,19]

In the case of acute myocardial infarction (AMI), FABP3 is released from the porous cell membranes of ischemic myocardial cells into circulation due to its small size.[12,20] Therefore, FABP3 is used as a biochemical marker in the early diagnosis of AMI and acute coronary syndrome[12,20], and can provide even better sensitivity than other commonly used markers including cTnI, CK-MB, and myglobin 0–6 hours after the onset of chest pain.[21] However, the concentration of FABP3 is affected by age and renal condition.[12,19] Also, special attention needs to be addressed to additional analytical issues, such as optimum cut-off value.[13,41–43]

Medix Biochemica offers three different monoclonal antibodies for the detection of human FABP3 (2302, 2303, and 2304). All of the antibodies are specific for FABP3 and show no cross-reactivity with FABP1 and FABP4. Additionally, Medix Biochemica has one recombinant FABP3 antigen in the product selection.

Anti-human FABP3 monoclonal antibodies and recombinant antigen

FABP3 antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
2302	100292	5	18	IgG_1	ELISA, LF, IT
2303	100293	5	24	IgG_1	ELISA, LF, IT
2304	100294	5	24	IgG_1	ELISA, LF, IT

FABP3 antigen	Product code
Recombinant FABP3, 100 µg	610043

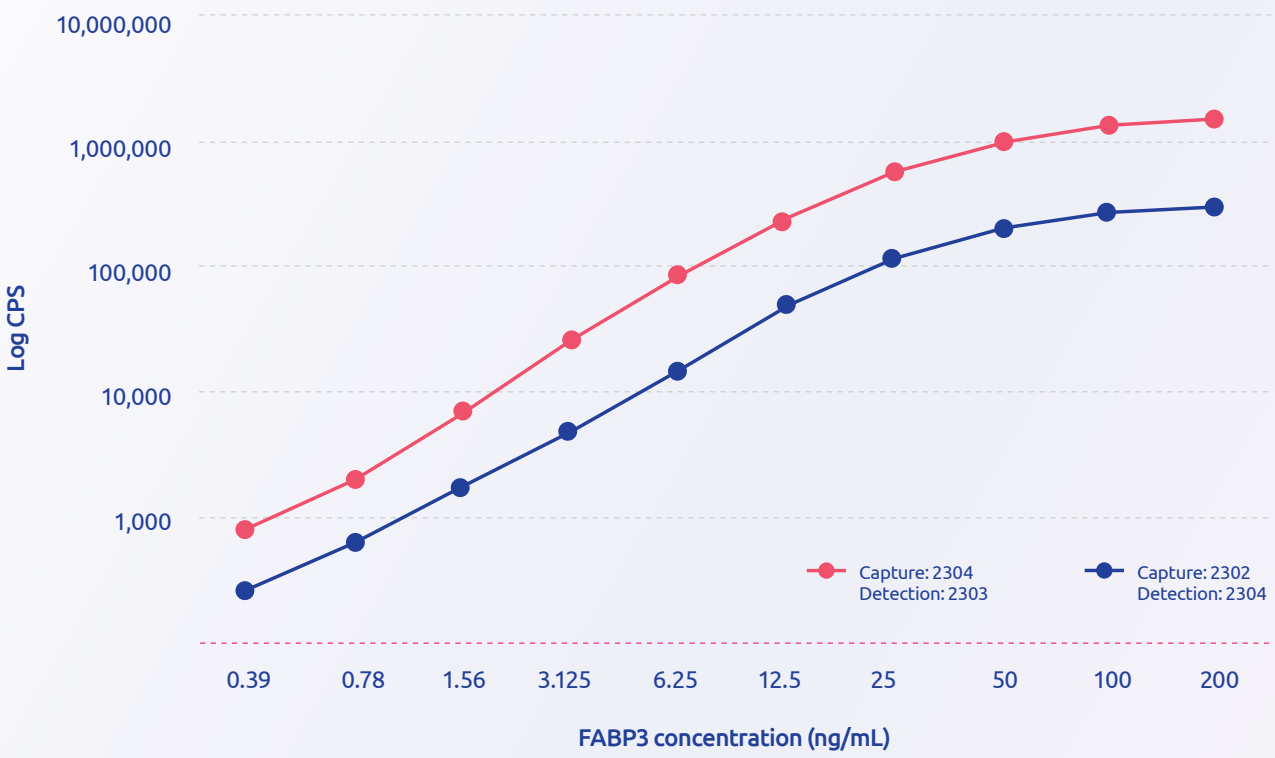
Pair recommendations

		Detection		
		2302	2303	2304
Capture	2302	–	–	+
	2303	–	–	+
	2304	+	+	–

Kinetic parameters

FABP3 antibody	Association rate constant, k_{on}	Dissociation rate constant, k_{off}	Affinity constant, K_A
2302	2.9×10^5 1/Ms	4.5×10^{-3} 1/Ms	$K_A = 6.5 \times 10^7$ 1/M, $K_D = 1.6 \times 10^{-8}$ M = 16 nM
2303	4.0×10^5 1/Ms	1.6×10^{-3} 1/Ms	$K_A = 2.5 \times 10^8$ 1/M, $K_D = 4.1 \times 10^{-9}$ M = 4.1 nM
2304	3.5×10^5 1/Ms	1.2×10^{-4} 1/Ms	$K_A = 2.8 \times 10^9$ 1/M, $K_D = 3.5 \times 10^{-10}$ M = 0.35 nM

FABP3 standard curves



Copeptin

Copeptin, which is a 39-aminoacid glycopeptide, forms the C-terminal part of pre-provasopressin (pre-proAVP). Pre-proAVP is a precursor protein synthesized in hypothalamus and consists of a signal peptide, arginine vasopressin (AVP, also known as antidiuretic hormone [ADH]), neurophysin II and copeptin. The components of pre-proAVP are cleaved during axonal transportation from hypothalamus to pituitary gland.[44]

AVP is released into the bloodstream in response to changes in plasma osmolarity and reduced cardiac output. However, it is unstable in isolated plasma and thus cannot be used as a biomarker. As copeptin is co-synthesized with AVP and is found in equimolar amounts with AVP in the bloodstream, and because it is stable in plasma for days it can be used as a surrogate biomarker of AVP release.[34] Several trials have assessed the diagnostic and prognostic value of copeptin in various cardiovascular diseases, especially in acute coronary syndromes (ACS) and heart failure.[45]

Copeptin is a promising biomarker for improving the diagnostics of ACS when used in combination with troponins.[35] Using copeptin in combination with cardiac troponin I (cTnI), for example, allowed safely ruling out acute myocardial infarction with a negative predictive value of over 99% in patients presenting with suspected ACS.[36] In addition, copeptin can potentially be used to predict outcomes of acute and chronic heart failure.[46–47]

Medix Biochemica has four monoclonal antibodies (4801, 4802, 4804, and 4806) for the detection of copeptin.

Anti-human copeptin monoclonal antibodies

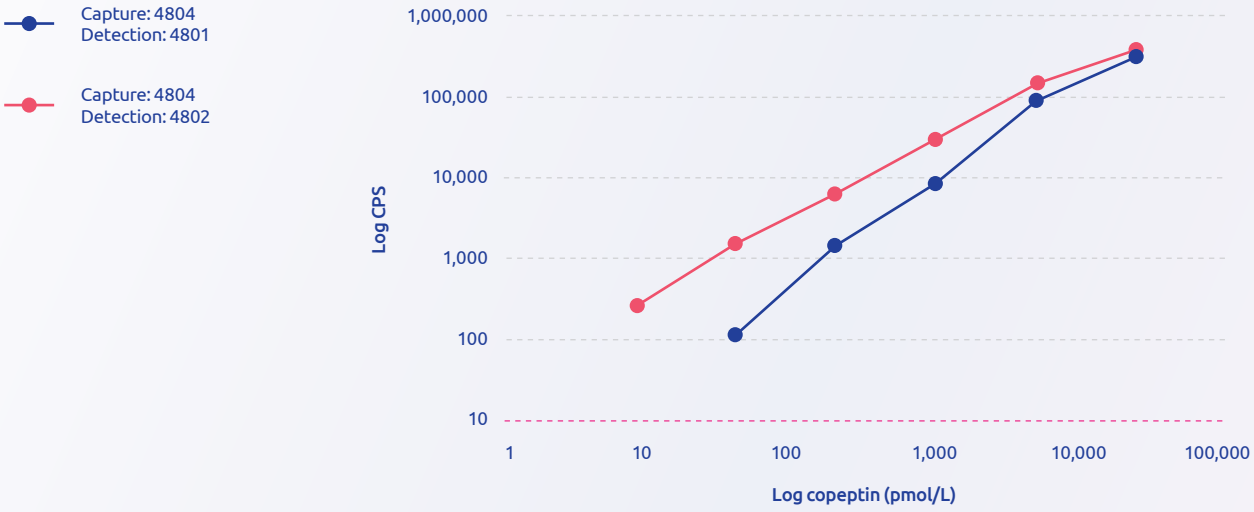
Copeptin antibody	Product code	Concentration (mg/mL)	Subclass	Applications tested
4801	100638	5	IgG_1	ELISA
4802	100639	5	IgG_1	ELISA
4804	100649	5	IgG_1	ELISA
4806	100648	5	IgG_1	ELISA

Pair recommendations

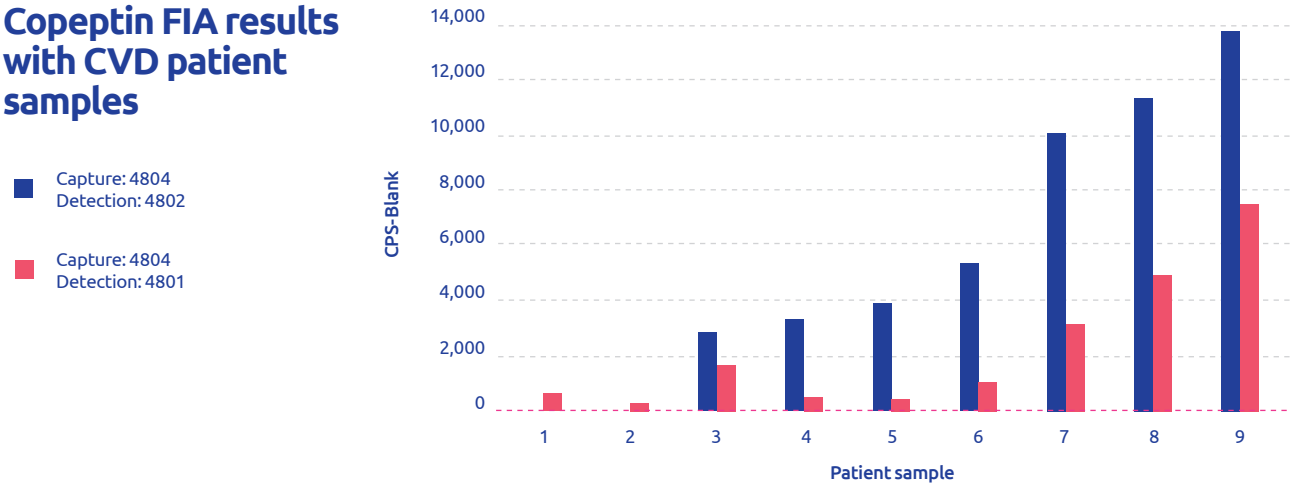
		Detection			
		4801	4802	4804	4806
Capture	4801	–	–	+	+
	4802	–	–	+	+
	4804	+	+	–	–
	4806	+	+	–	–

Binding epitopes

Copeptin standard curves



Copeptin FIA results with CVD patient samples



Myoglobin

Myoglobin is an oxygen-binding cytosolic heme protein and belongs to the globin family together with hemoglobin and neuroglobin. Globin-family proteins have a characteristic globin fold consisting of eight alpha-helices and a heme group. Myoglobin is found in oxidative striated muscles and cardiac myocytes as well as in smooth muscle cells acting as an oxygen storage depot.[48]

Myoglobin is a sensitive marker for muscle damage, and is rapidly released after acute myocardial injury (AMI).[23] Myoglobin is the earliest marker to rise after AMI, and appears in blood 1–3 hours post AMI, reaches its maximum in 4–7 hours and goes back to baseline after 24–36 hours.[26] However, because of its rapid kinetics, the use of myoglobin as a biomarker may miss patients who do not show early signs of infarction.[23] In addition, myoglobin is less heart-specific than for example CK-MB and FABP3[27], and has limited specificity for patients with renal insufficiency and skeletal muscle injury.[49,50,52]

Currently, Medix Biochemica offers three monoclonal antibodies (7001, 7004, and 7005) for the specific detection of myoglobin. In addition, Medix Biochemica offers a recombinant myoglobin antigen.

Anti-human myoglobin monoclonal antibodies and recombinant antigen

Myoglobin antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
7001	100378	5	24	IgG_{2b}	ELISA, LF, IT
7004	100354	5	24	IgG_1	ELISA, LF, IT
7005	100078	5	36	IgG_1	ELISA, LF, IT

Myoglobin antigen	Product code
Recombinant Myoglobin, 100 µg	610030

Pair recommendations

		Detection		
		7001	7004	7005
Capture	7001	–	+	–
	7004	+	–	+
	7005	+	+	–

Kinetic parameters

Myoglobin antibody	Affinity constant, K_A
7001	1×10^8 1/M
7004	7×10^9 1/M
7005	1×10^9 1/M

CK-MB

Creatine kinase (CK, also known as creatine phosphokinase or phospho-creatine kinase) is a member of a highly conserved family of phosphoryl transfer enzymes called phosphagen (guanidino) kinases and is expressed in various cells and tissue types. CK catalyzes the reversible transfer of phosphate from phosphocreatine to ADP to yield ATP and creatine.[22] CK consists of two subunits, which can be either the M (muscle) or B (brain) type. In humans, there are three different isoenzymes, BB, MM, and MB.[12,23] The CK-MB isoenzyme, also known as CK-2, is predominantly found in the heart muscle, and serves as a biomarker for cardiac muscle injury.[13,51,52]

CK-MB is present in high concentration uniquely in the myocardium, but it can be found in smaller concentrations in skeletal muscle and the brain. The levels of CK-MB are normally very low or undetectable in the blood, but increase rapidly in both heart and skeletal diseases with highest concentrations in the cardiac muscle (22% in the cardiac muscle compared to 1–3 % in the skeletal muscle). Measurement of CK-MB concentration in plasma or serum is used as a tool for the diagnosis of acute myocardial infarction (AMI), and is routinely determined in emergency patients. CK-MB measurement is especially valuable in the 20% of AMI patients that are clinically asymptomatic.[13,49,50]

Medix Biochemica has two monoclonal antibodies (7501 and 7502) against human creatine kinase MB isozyme. The antibody 7502 is specific for CK-MB isoform, with less than 10% cross-reactivity to isoforms CK-BB or CK-MM.

Anti-human CK-MB monoclonal antibodies

CK-MB antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
7501	100630	5	24	IgG_1	ELISA
7502	100086	5	18	IgG_1	ELISA

Pair recommendations

		Detection
		7502
Capture	7501	+

Kinetic parameters

CK-MB antibody	Affinity constant, K_A
7501	2×10^9 1/M
7502	1×10^8 1/M

MPO

Myeloperoxidase (MPO) is an enzyme that belongs to the heme-peroxidase superfamily. It is highly expressed in neutrophils and monocytes and released during their activation. Accordingly, MPO is involved in the antimicrobial innate immune response.[28]

MPO has a 150-kDa homodimeric structure that consists of two 15-kDa light chains and two variable-weight heavy chains bound to a heme group.[29] It catalyzes the conversion of hydrogen peroxide to hypochlorous acid, which has strong anti-microbial and detoxification properties but can also cause oxidative damage to the host tissue. In addition to playing a role in the host immune response, MPO has been identified in atheromatous plaques[30] and can exert several proatherogenic effects. These include oxidation of low-density lipoprotein (LDL) and high-density lipoprotein (HDL)[31] as well as induction of vascular dysfunction through reducing nitric oxide bioavailability.[32]

Several studies have indicated that MPO could be used as a cardiovascular disease risk marker along with traditional markers, especially in patients with unstable coronary artery disease.[31]

Medix Biochemica provides three high-quality monoclonal antibodies (1701, 1702, and 1703) against MPO.

Anti-human MPO monoclonal antibodies

MPO antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
1701	100266	5	36	IgG_1	ELISA
1702	100267	5	36	IgG_1	ELISA
1703	100268	5	36	IgG_1	ELISA

Kinetic parameters

MPO antibody	Association rate constant, k_{on}	Dissociation rate constant, k_{off}	Affinity constant, K_A
1701	1×10^6 1/Ms	1×10^{-3} 1/s	$K_A = 1 \times 10^9$ 1/M, $K_D = 1 \times 10^{-9}$ M
1702	8×10^5 1/Ms	2×10^{-3} 1/s	$K_A = 5 \times 10^8$ 1/M, $K_D = 2 \times 10^{-9}$ M
1703	9×10^5 1/Ms	2×10^{-4} 1/s	$K_A = 5 \times 10^9$ 1/M, $K_D = 2 \times 10^{-10}$ M

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CPS = Counts per second
CLIA = Chemiluminescence immunoassay
ELISA = Enzyme-linked immunosorbent assay
FIA = Fluoroimmunoassay
IT = Immunoturbidimetry
LF = Lateral flow
N/A = Not Applicable
N/D = Not Determined