TechNotes

HyTest

Clinical and Research Area | Cardiac Markers

Cardiac troponin T (cTnT)

The cardiac isoform of TnT is, similarly to cTnI, widely used as a marker of myocardial cell injury. cTnT has the same release kinetics into the bloodstream and the same sensitivity for minor myocardial injury as cTnI.

In human beings, cardiac troponin T is encoded by the TNNT2 gene. The major isoform found in normal adult human heart tissue (isoform 6 or TnT3) is 287 amino acids long with a calculated molecular weight of 34.6 kDa.

CLINICAL UTILITY

✓ Early marker of acute myocardial infarction

Reagents for immunoassay development

We provide MAbs that are suitable for the development of immunoassays for diagnostic purposes as well as several MAbs that are recommended for research use (see Figure 1). We also provide polyclonal anti-cTnT antibodies as well as purified native and recombinant human cTnT and recombinant human slow and fast skeletal TnT proteins. The skeletal proteins are ideal for studying immunoassay cross-reactivity to these isoforms.

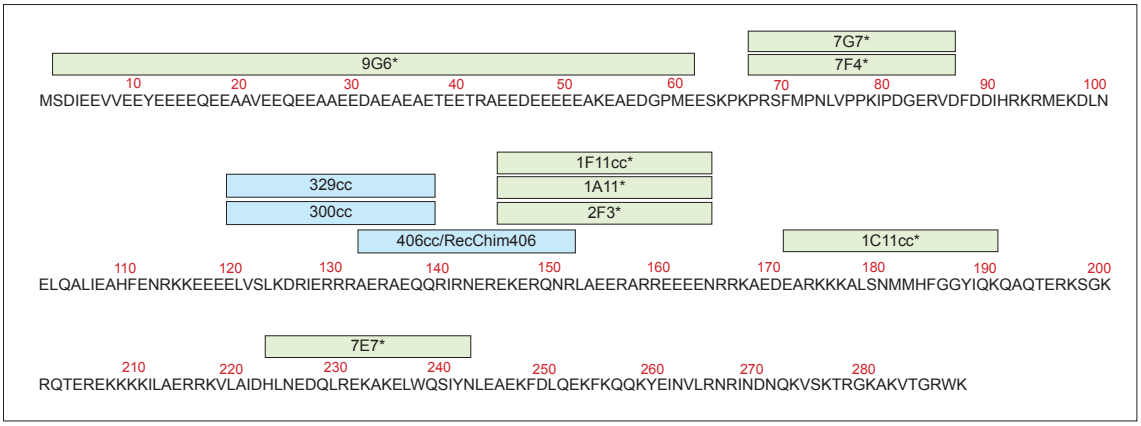


Figure 1. Epitope mapping of HyTest anti-cTnT monoclonal antibodies. We offer antibodies for the development of high-sensitivity cTnT assays (blue) as well as for research purposes (green, marked with *).

Monoclonal antibodies for high-sensitivity cTnT assays

We have developed three anti-cTnT MAbs (300cc, 329cc and 406cc; Cat.# 4T19cc) that can be used for the development of an immunoassay with superior sensitivity (limit of detection better than 0.3 ng/l) and high specificity (no cross-reaction to cTnI or to skeletal isoforms of TnT up to 30 µg/l). MAb 406cc is also available as a recombinant chimeric construct in which the original wild type variable domains of the antibody and human IgG1 constant domains are combined (Cat.#RC4T19, MAb RecChim406). In our preliminary tests, RecChim406 is slightly more sensitive than 406cc (see Figure 5).

The ability of the antibody pairs 329cc-406cc and 406cc-300cc to recognize cTnT in the blood of AMI patients has been studied with over 80 serum and plasma samples. The antibody pairs demonstrate a good correlation with a commercially available hs-cTnT assay. Results of the analysis of 38 serum samples are provided in Figure 2.

Negligible cross-reactivity to skTnT

In high-sensitivity troponin assays, the specificity of the antibodies utilized is of utmost importance as even minor cross-reactivities could result in false positives.

We investigated the cross-reactivity of MAbs 300cc, 329cc and 406cc to skeletal isoforms of troponin T. First, individual MAbs were incubated with purified native cTnT and a mixture of recombinant slow and fast skTnT. All MAbs recognized only cTnT (see Figure 3). Second, we tested the cross-reactivity of the two prototype assays to purified native skTnT, recombinant fast skTnT and recombinant slow skTnT. IGFBP-4 and MPO antigens were used as negative controls. Also in this case the cross-reactivity was well below 0.1% (see Figure 4).

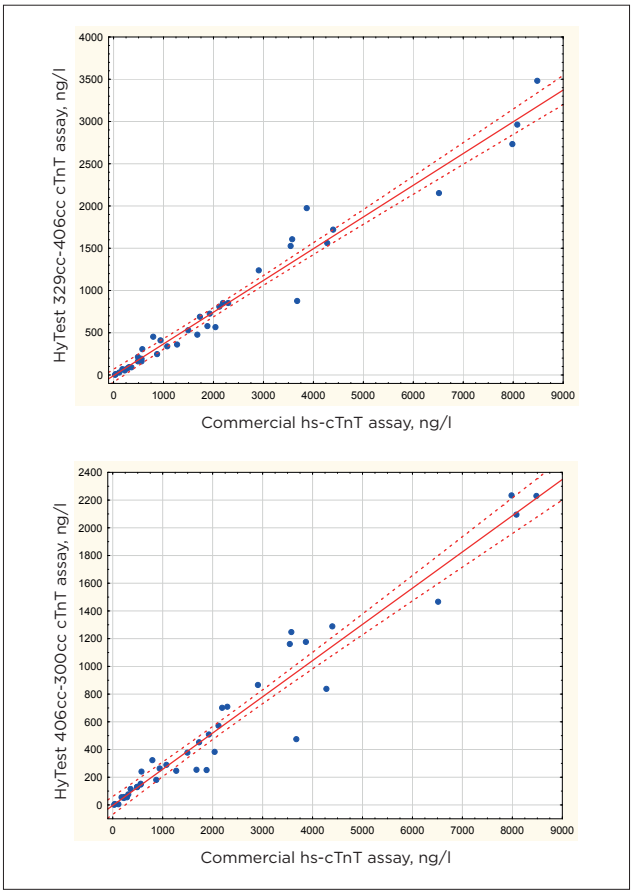


Figure 2. HyTest immunoassays show good correlation to a commercially available hs-cTnT assay. The concentration of cTnT in 38 serum samples obtained from AMI patients was determined by using two immunoassays that utilized HyTest antibodies (capture-detection pairs 329cc-406cc and 406cc-300cc) and a commercially available hs-cTnT assay.

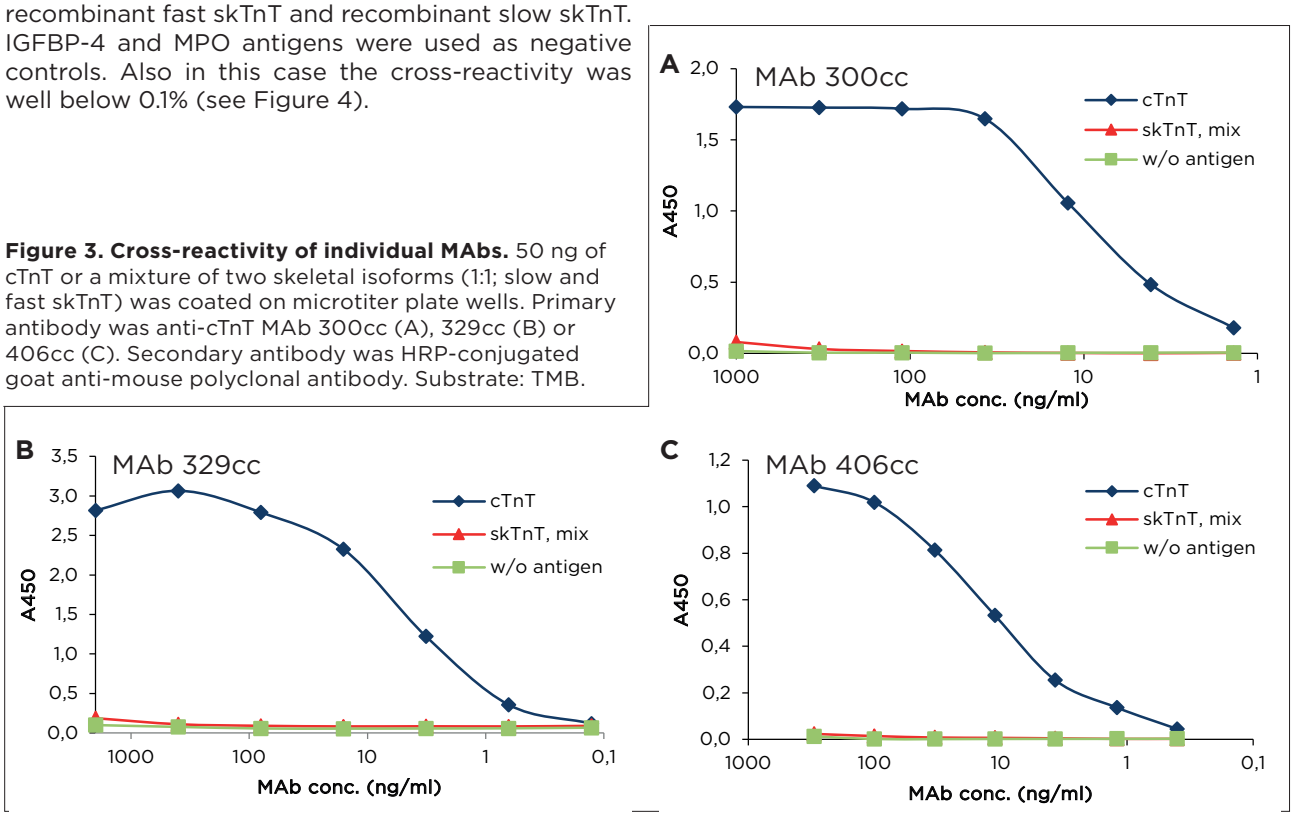
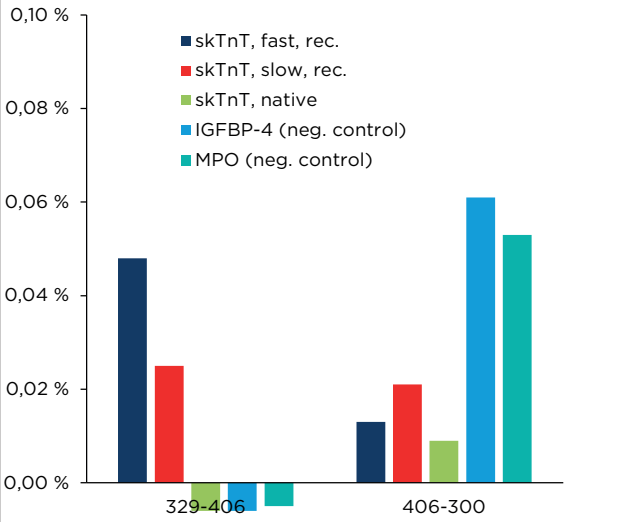


Figure 3. Cross-reactivity of individual MAbs. 50 ng of cTnT or a mixture of two skeletal isoforms (1:1; slow and fast skTnT) was coated on microtiter plate wells. Primary antibody was anti-cTnT MAb 300cc (A), 329cc (B) or 406cc (C). Secondary antibody was HRP-conjugated goat anti-mouse polyclonal antibody. Substrate: TMB.



Antigen	329cc-406cc	406cc-300cc
cTnT	100 %	100 %
fast skTnT, rec.	0.048 %	0.013 %
slow skTnT, rec.	0.025 %	0.021 %
skTnT, native	-0.006 %	0.009 %
IGFBP-4 (neg. control)	-0.006 %	0.061 %
MPO (neg. control)	-0.005 %	0.053 %

Figure 4. Cross-reactivity of prototype assays 329cc-406cc and 406cc-300cc. Reactivity to various antigens (100 ng/ml) was investigated in sandwich immunoassays. Both assays demonstrated negligible cross-reactivity to all markers tested. In hs-cTnT immunoassays the cross-reactivity should be < 0.10 % and preferably < 0.05-0.03 %.

Antibodies for research purposes

We offer several MAbs that are recommended for research purposes. They also cross-react with cTnT proteins from different animal species (see Table 1).

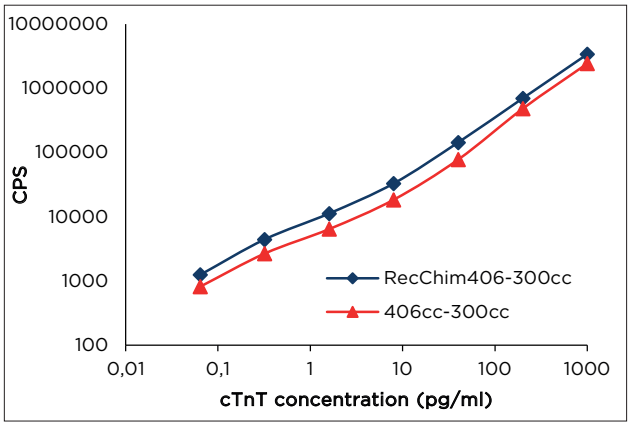


Figure 5. Comparing the performance of MAb 406cc and RecChim406 using a CLIA assay. In both assays, 406 was used as the coating MAb and biotin labeled 300cc as the detection MAb.

Purified antigens

Native human cTnT

HyTest cTnT (Cat.# 8T13) is purified from human cardiac muscle tissue by immunoaffinity chromatography followed by an additional ion exchange chromatography step.

Recombinant human cTnT

Isoform 6 (which is also known in the literature as TnT3) is the major isoform of troponin T that is presented in normal adult human heart tissue.

Our recombinant human cTnT (Cat.# 8RTT5) is produced in *E. coli* by expressing a gene encoding for the 288 amino acid long isoform 6 (TnT3) of cTnT. This isoform is the main isoform of cTnT in normal adult human heart tissue. The protein has an additional Met residue at its N-terminus.

Recombinant human slow and fast skTnT

The recombinant slow skeletal TnT (Cat.# 8RST2) and fast skeletal TnT (Cat.# 8RFT4) are ideal for studying immunoassay cross-reactivity to these isoforms.

TABLE 1. Cross-reactivity of anti-cTnT MAbs with antigens from different animal species in Western blotting.

MAb	Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
7F4	++	N/A	++	N/A	-	-	-	N/A	N/A	-
7G7	+	+	-	-	-	-	-	-	-	-
2F3	++	+	++	++	+	+	+	+	+	+
1A11	++	++	++	++	+	+	+	+	++	+
1F11	++	++	++	++	+	+	+	+	+	+

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Troponin T cardiac	4T19	9G6	IgG1	EIA, WB, a.a.r. 2-61
		7F4	IgG2b	EIA, WB, a.a.r. 67-86
		7G7	IgG1	EIA, WB, a.a.r. 67-86
		2F3	IgG2b	EIA, WB, a.a.r. 145-164
		1A11	IgG2b	EIA, WB, a.a.r. 145-164
		1F11	IgG2b	EIA, WB, a.a.r. 145-164
		1C11	IgG1	EIA, WB, a.a.r. 171-190
		7E7	IgG1	EIA, WB, a.a.r. 223-242
	4T19cc	300cc	IgG1	*In vitro*, EIA, a.a.r. 119-138
		329cc	IgG1	*In vitro*, EIA, a.a.r. 119-138
		406cc	IgG2a	*In vitro*, EIA, a.a.r. 132-151
		1F11cc	IgG2b	*In vitro*, EIA, WB, a.a.r. 145-164
		1C11cc	IgG1	*In vitro*, EIA, WB, a.a.r. 171-190
	RC4T19	RecChim406	IgG1	EIA, recombinant chimeric antibody

HUMAN ANTIGENS

Product name	Cat. #	Purity	Source
Troponin T cardiac, human	8T13	>98%	Human cardiac muscle
Troponin T cardiac, human, recombinant	8RTT5	>95%	Recombinant
Troponin T skeletal muscle, human	8T24	>95%	Human skeletal muscle
Troponin T fast skeletal, human, recombinant	8RFT4	>95%	Recombinant
Troponin T slow skeletal, human, recombinant	8RST2	>95%	Recombinant
Troponin complex (I-T-C), human	8T62	N/A	Human cardiac muscle
Troponin complex (I-T-C), artificial	8T62a	N/A	Human cardiac muscle

ANIMAL ANTIGENS

Product name	Cat. #	Purity	Source
Troponin T cardiac, bovine	8T13b	>98%	Bovine cardiac muscle
Troponin T cardiac, canine	8T13c	>98%	Canine cardiac muscle
Troponin T cardiac, mouse	8T13m	>98%	Mouse cardiac muscle
Troponin T cardiac, porcine	8T13p	>98%	Porcine cardiac muscle
Troponin T cardiac, rat	8T13r	>98%	Rat cardiac muscle
Troponin T skeletal muscle, bovine	8T24b	>95%	Bovine skeletal muscle
Troponin T skeletal muscle, canine	8T24c	>95%	Canine skeletal muscle
Troponin T skeletal muscle, mouse	8T24m	>95%	Mouse skeletal muscle
Troponin T skeletal muscle, porcine	8T24p	>95%	Porcine skeletal muscle
Troponin T skeletal muscle, rat	8T24r	>95%	Rat skeletal muscle

HyTest Ltd
Intelligate 1, 6th floor, Joukahaisenkatu 6
FI-20520 Turku, FINLAND

Tel. +358 2 512 0900, Fax +358 2 512 0909
E-mail: hytest@hytest.fi
www.hytest.fi