

Cardiac troponin I (cTnI)

Troponin complex is a heteromeric protein complex that plays an important role in the regulation of skeletal and cardiac muscle contraction. The complex consists of three subunits: troponin I (TnI), troponin T (TnT) and troponin C (TnC).

Cardiac troponin I is currently considered as the gold standard biomarker test for myocardial infarction. Moreover, cTnI measurements by new generation of high-sensitivity cTnI assays could be helpful for long-term risk stratification of different patient groups including patients with heart failure or acute coronary syndrome.

At HyTest we have intensively studied troponin I for over 20 years. Based on this research we constantly aim at developing improved antibodies to be used in immunoassays needed in accurate cardiac disease diagnostics. We have generated and tested several thousand monoclonal antibodies specific to different regions of cTnI molecule and tested numerous different mAb combinations in order to find the best pairs for a precise and sensitive cTnI immunoassay.

In 2004, HyTest's troponin I-T-C complex was selected by the American Association for Clinical

Chemistry Standardization Subcommittee to be used by assay manufacturers as reference material in troponin I assays. The certified reference material (SRM 2921) is available only from the National Institute of Standards and Technology.

CLINICAL UTILITY

- ✓ Acute myocardial infarction (AMI)
- ✓ Unstable angina
- ✓ Cardiac muscle injury and cell death

REAGENTS FOR ASSAY DEVELOPMENT

- ✓ Monoclonal antibodies for cTnI
- ✓ Polyclonal antibodies for cTnI
- ✓ Native human cardiac troponin I products
- ✓ Native human cardiac troponin complex
- ✓ Artificial troponin complexes
- ✓ cTnI free serum
- ✓ Tools to study assay susceptibility to cTnI modifications
- ✓ cTnI calibrator set
- ✓ Troponin T and C antibodies and antigens
- ✓ Native troponin antigens from several animal species

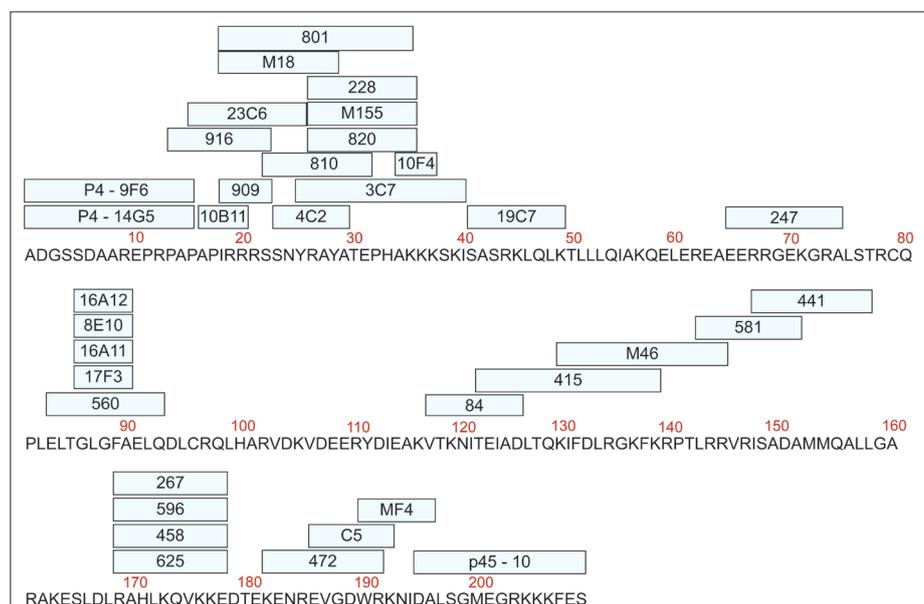


Figure 1. Epitope mapping of HyTest anti-cTnI monoclonal antibodies. We offer more than 30 specially selected antibodies specific for various epitopes along the cTnI molecule.

Factors influencing epitope recognition by antibodies

The most common reason for the discrepancy in the cTnI assay measurements is the difference in the epitope specificity of the antibodies used in various assays. Due to several posttranslational modifications and presence of autoantibodies in clinical samples, it is critical to carefully validate the performance of antibodies in order to achieve reliable, quantitative detection of the biomarker in blood samples.

Factors influencing cTnI immunodetection

- ✓ Proteolytic degradation of cTnI
- ✓ Phosphorylation status of cTnI
- ✓ Complex formation between troponin I, C and T
- ✓ Presence of heparin in samples
- ✓ Autoantibodies
- ✓ mAb cross-reaction with skeletal troponin I

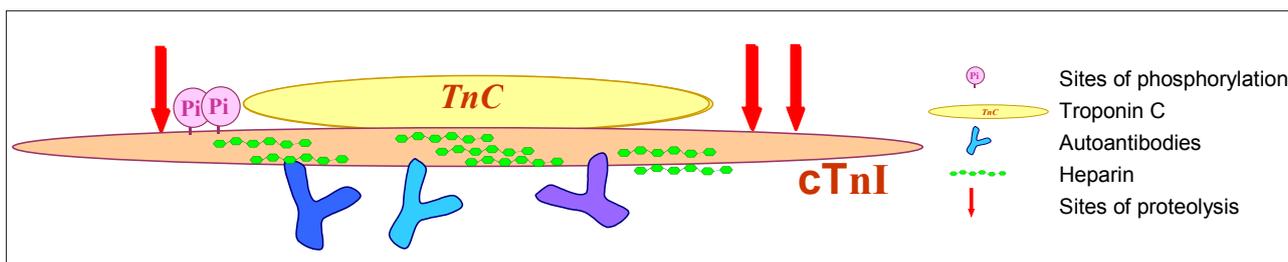


Figure 2. Factors influencing cTnI immunodetection.

Antibodies for high-sensitivity cTnI immunoassays

When designing a sensitive and precise immunoassay it is important to consider the effect of all the factors influencing biomarker detection to minimize bias in the assay. Factors that influence cTnI measurements are schematically presented in Figure 2. Antibodies specific to different parts of the molecule are sensitive to these factors in different degrees. For instance, it is well-known that purified cTnI is highly susceptible to proteolytic degradation. However, in troponin complex the central part of the cTnI closely interacts with TnC which protects cTnI from proteolytic degradation. Consequently, the epitopes located on the central part of the cTnI are significantly more stable than the epitopes located at the terminal parts of the molecule (see Figure 3). On the other hand, not every antibody specific to the central part of the molecule can recognize cTnI in patient's blood. This is due to the fact that in blood cTnI is complexed with TnC and TnC covers some epitopes located in that region.

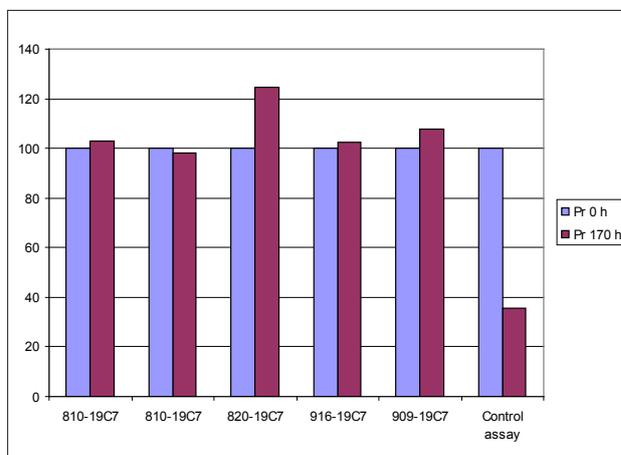


Figure 3. Effect of proteolytic degradation. Antibodies specific to the fragment 13-36 of the cTnI molecule are insensitive to proteolytic degradation of the cTnI and were used here as capture mAbs together with mAb 19C7, that is also specific to the stable cTnI region (epitope 41-49). The antigen was native troponin complex (Pr 0 h) or native troponin complex incubated with endogenous tissue proteases for 170 hours (Pr 170 h). In the control assay, two mAbs specific to the N- and C-terminal parts of cTnI were used.

In an immunoassay, the limit of detection is dependent on good antibodies but also on the features of the platform. For example, utilizing HyTest anti-cTnI antibodies in LamdaGen's high-sensitivity cTnI plasmonic ELISA the limit of detection was shown to be 0.64 pg/ml¹ (see Figure 4).

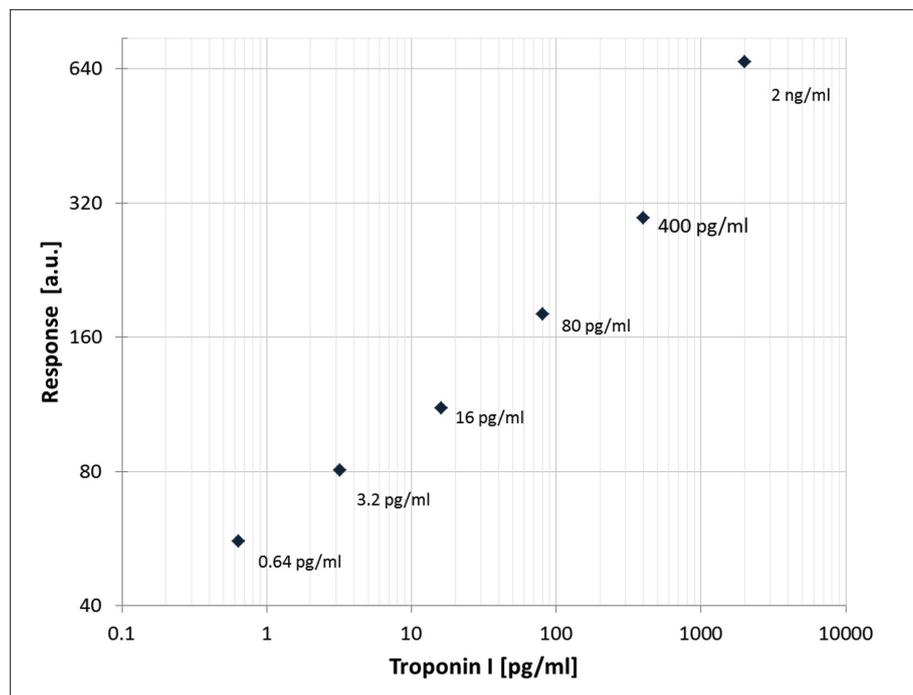


Figure 4. Highly sensitive quantitation of cTnI in 87% FBS with LamdaGen's plasmonic ELISA utilizing HyTest mAbs. Dose response curve was obtained by spiking cTnI into 87% fetal bovine serum. Each data point represents the average of five independent measurements. Reprinted with permission from LamdaGen Corp.

Troponin I research at HyTest

HyTest R&D scientists have intensively studied troponin I for over 20 years and significantly contributed to development of reliable quantitative immunoassays for cTnI.

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¹OES™ quantification of cTnI in whole serum. Application Note, 2013. LamdaGen Corporation. www.LamdaGen.com

TechNotes | Cardiac troponin I (cTnI)

Monoclonal antibodies:

Cat. #	Product	Recognizes	Tested applications
4T21	Monoclonal mouse anti-cardiac troponin I (cTnI) Note. Several different mAbs separately available under Cat.# 4T21	• Endogenous cTnI in human blood samples	• Immunoassays • Western blotting • Affinity purification • Immunoprecipitation • Immunohistochemistry
4T45	Monoclonal mouse anti-cardiac troponin I (cTnI), phosphorylated form	• Phosphorylated form of cTnI	• ELISA • Western blotting
4T46	Monoclonal mouse anti-cardiac troponin I (cTnI), dephosphorylated form	• Dephosphorylated form of cTnI, no cross-reaction with mono- or biphosphorylated cTnI	• ELISA • Western blotting
4TC2	Monoclonal mouse anti-human native cardiac troponin complex	• Native cardiac troponin complex, no cross-reaction with individual components of troponin complex	• Human cardiac Tn complex immunodetection in direct ELISA • High sensitivity cTnI sandwich immunoassay in pairs with antibodies specific to human cTnI (Cat.# 4T21) and TnC (Cat.# 4T27)
4T19	Monoclonal mouse anti-cardiac troponin T (cTnT) Note. Several different mAbs separately available under Cat.# 4T19	• Endogenous cTnT in human blood samples	• Immunoassays • Western blotting • Affinity purification
4T27	Monoclonal mouse anti- troponin C (TnC) Note. Few different mAbs separately available under Cat.# 4T27	• Endogenous TnC in human blood samples	• Immunoassays • Western blotting • Affinity purification

Antigens:

Cat. #	Product	Source	Purity
8T53	Human cardiac TnI	Human cardiac muscle	>98%
8T53ph	Human cardiac TnI (phosphorylated)	Human cardiac muscle	>95%
8T53dp	Human cardiac TnI (dephosphorylated)	Human cardiac muscle	>95%
8T62	Human cardiac troponin complex (I-T-C)	Human cardiac muscle	N/A
8T62a	Artificial I-T-C complex	Proteins purified from human cardiac muscle	N/A
8IC63	Artificial I-C complex	Proteins purified from human cardiac muscle	N/A
8T25	Human skeletal TnI	Human skeletal muscle	>95%
8T13	Human cardiac TnT	Human cardiac muscle	>98%
8T24	Human skeletal TnT	Human skeletal muscle	>95%
8T57	Human TnC	Human cardiac muscle	>98%

Serum and other products:

Cat. #	Product	Source/Remarks
8TFS	cTnI free serum	Pooled normal human serum
K01	Troponin I Diversity Kit	Different forms of human cTnI
8T60	Troponin I Calibrator set	Troponin complex in normal human serum