TechNotes

HyTest

Clinical and Research Area | Cardiac Markers

Recombinant cardiac troponin ITC and IC complexes

Troponin complex is a complex of proteins that participates in the regulation of muscle contraction. It consists of troponin I (TnI), troponin T (TnT), and slow skeletal/cardiac troponin C (TnC), which are associated in the ratio of 1:1:1 in a functionally active unit (1). In the heart muscle cells, troponin I and troponin T are presented by the specific cardiac isoforms cTnI and cTnT, respectively. This in turn makes it possible to use them as specific biomarkers of myocardial infarction and cardiovascular diseases (1).

Troponin complex as a marker in diagnostics

Acute myocardial infarction (AMI) causes the damage of heart muscle and the release of cardiac troponin complex into the blood of patients where it can be detected by measuring its components: either cTnI or cTnT using cTnI or cTnT assays, respectively (2). The fragments of cTnI and cTnT are also released from the damaged myocardium and they can be detected by the same assays. Both the cTnI and cTnT proteins are currently widely used as the guideline-recommended markers of AMI (3-6) and myocardial injury associated with postoperative myocardium trauma, chemotherapy cardiotoxicity, and many other diseases related to cardiac muscle injury.

Recombinant Troponin complexes

HyTest offers two recombinant Troponin complexes: cardiac troponin ternary complex (cTn ITC complex, Cat.# 8ITCR) and binary complex (cTn IC complex, Cat.# 8ICR3). Ternary troponin complex is constructed from recombinant cTnI, cTnT, and slow skeletal/cardiac TnC isoforms. All of the proteins are expressed in *E. coli*, purified, and then used to form troponin complex. The resulting complex is separated from free proteins and dimeric complex and shows a final purity of more than 95% (Fig 1).

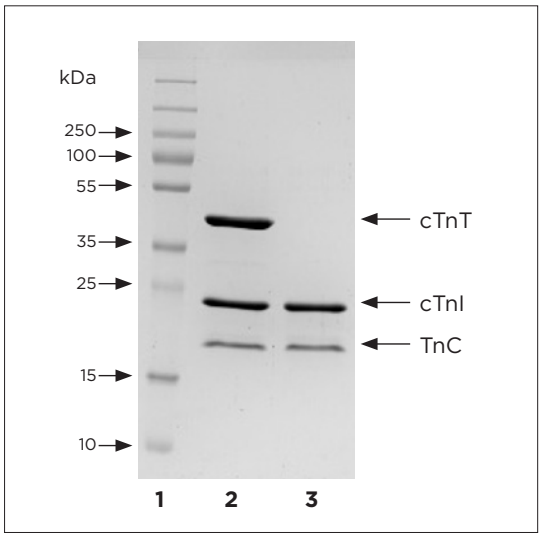

FIGURE 1. SDS-PAGE of recombinant cTn ITC complex and cTn IC complex under reducing conditions in a 10-20% gradient gel.
Lane 1: MW standards
Lane 2: recombinant cTn ITC complex
Lane 3: recombinant cTn IC complex.

CLINICAL UTILITY

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

The integrity of recombinant ternary troponin complex is confirmed by fluorescent immunoassay (FIA) using the Tcom8 (Cat.# 4TC2) - 7E7 (Cat.# 4T19) assay that can only detect troponin complex if all three proteins of the complex are bound together: the Tcom8 mAb recognizes cTnI and TnC bound to each other, and it does not recognize separate cTnI or TnC isoforms, while the mAb 7E7 recognizes cTnT. The HyTest recombinant troponin ternary complex is similar to the native troponin complex that is isolated from cardiac tissue (Fig. 2).

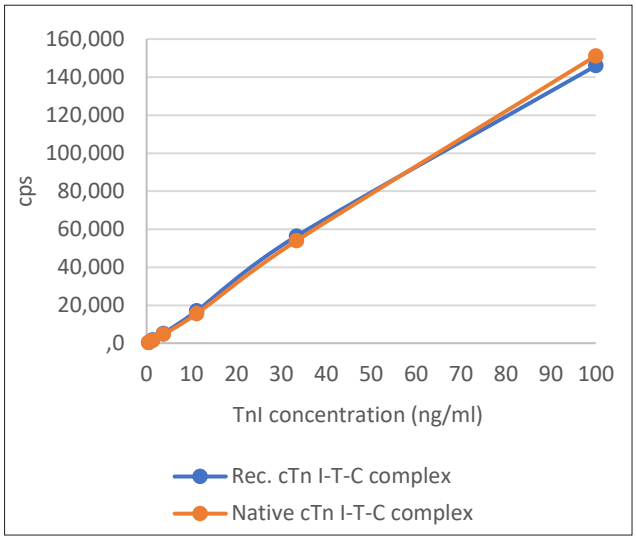

FIGURE 2. Comparison of recombinant cTn ITC and native cTn ITC complex using an in-house assay. The Tcom8 (Cat.# 4TC2) - 7E7 (Cat.# 4T19) assay can only detect cardiac troponin ternary complex if all three troponin isoforms (cTnI, cTnT and TnC) are bound together.

The HyTest recombinant binary troponin complex is constructed from recombinant cTnI and TnC. All of the proteins are expressed in *E. coli*, purified, and then used to form binary troponin complex. The resulting complex is separated from free TnC and shows final purity of more than 95% (Fig 1). Recombinant binary troponin complex is similar to the native binary troponin complex constructed from native cTnI and TnC (Fig. 3).

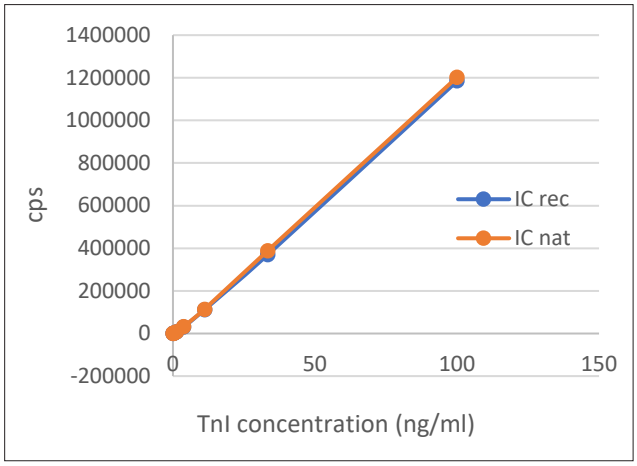

FIGURE 3. Comparison of recombinant cTn IC and native cTn IC complex using an in-house assay. The Tcom8 (Cat.# 4TC2) – 7B9cc (Cat.# 4T27cc) assay can only detect cardiac troponin binary complex if all two troponin isoforms (cTnI and TnC) are bound together.

The concentration of both recombinant ternary and binary troponin complex is given by the concentration of cTnI in the complex.

The use of recombinant Troponin complexes

Both recombinant troponin complexes can be used as a standard in immunoassays for the detection of cTnI in the blood of patients and as a cTnI calibrator. Recombinant ternary troponin complexes can also be used as a standard in immunoassays for the detection of cTnT in the blood of patients and as a cTnT calibrator.

Special information

Recombinant ternary and binary troponin complexes cannot be lyophilized and they should be stored as a solution at -70 °C. Both complexes tolerate up to five freeze-thaw cycles with a change in activity of less than 10%, although it is recommended to avoid freeze-thaw cycles.

Ordering information

ANTIGEN

Product name	Cat. #	Purity	Source
Troponin IC complex, cardiac, human, recombinant	8ICR3	>95%	Recombinant
Troponin ITC complex, cardiac, human, recombinant	8ITCR	>95%	Recombinant

References

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2. **Christenson R, Azzazy H.** Biochemical markers of the acute coronary syndromes. Clin Chem 1998; 44:1855–64.
3. Myocardial infarction redefined – A consensus document of The Joint European Society of Cardiology/ American College of Cardiology Committee for the Redefinition of Myocardial Infarction Eur. Heart J. 2000, 21(18): 1502-1513.
4. **Thygesen, K et al.** Third universal definition of myocardial infarction. Circulation. 2012, 126: 2020- 2035.
5. **Sandoval, Y and Thygesen, K.** Myocardial Infarction Type 2 and Myocardial Injury. Clin Chem. 2017, 63(1):101-107.

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