

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 three recombinant Troponin complexes: cardiac troponin ternary complex (cTn ITC complex, Cat.# 8ITCR), binary complex (cTn IC complex, Cat.# 8ICR3), and a chimeric binary complex that consists of a most stable part of cTnI (fragment 28-110) that is connected with TnC by a 20-aa flexible linker of cTn I-C complex (Cat.# 8IFC20). 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).

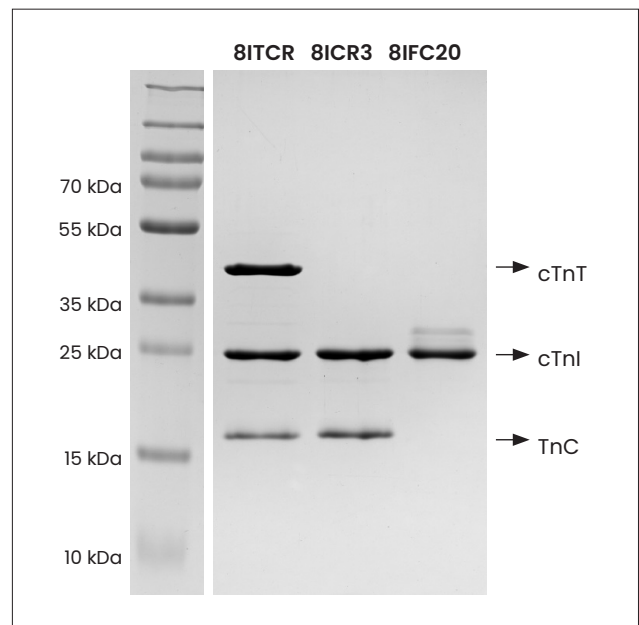


Figure 1. SDS-PAGE of recombinant cTn ITC complex, cTn IC complex and recombinant chimeric cTnI (a.a.r. 28-110)-C complex under reducing conditions in a 10-20% gradient gel.

Lane 1: MW standards

Lane 2: recombinant cTn ITC complex, Cat.# 8ITCR

Lane 3: recombinant cTn IC complex, Cat.# 8ICR3

Lane 4: recombinant chimeric cTn I (a.a.r. 28-110)-C complex Cat.# 8IFC20

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).

The immunoreactivity of all developed recombinant troponin complexes was comparable with the native analogue in two-step sandwich assays. The test panel include five antigens, native cTn ITC complex (ITCnat), binary cTn IC complex assembled from native proteins (ICart), recombinant ternary cTn ITC complex (8ITCR), recombinant binary cTn IC complex (8ICR3) and recombinant chimeric binary complex (8IFC20). Based on the test

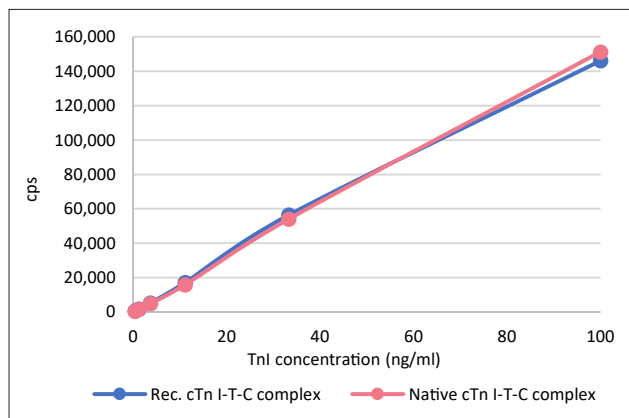


Figure 2. Comparison of recombinant cTn ITC and native cTn ITC complex using an in-house assay. The Tcom8 (Cat.# 4TC2) - 7E7 (Cat.# 4T19) assay can only detect cardiac troponin ternary complex if all three troponin isoforms (cTnI, cTnT and TnC) are bound together.

REFERENCES

1. **Gomes, AV et al.** The role of Troponin in muscle contraction. *Life*. 2002, 54: 323-333.
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

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
Troponin I (fragment 28–110) – troponin C complex, cardiac, human, recombinant chimeric	8IFC20	>95%	Recombinant

results (Fig. 3), there is no significant difference when comparing the recombinant troponin complexes with the native protein.

The concentration of all three troponin complexes is given by the concentration of cTnI in the complex.

The use of recombinant Troponin complexes

The three 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

All three troponin complexes cannot be lyophilized and they should be stored as a solution at -70 °C. These 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.

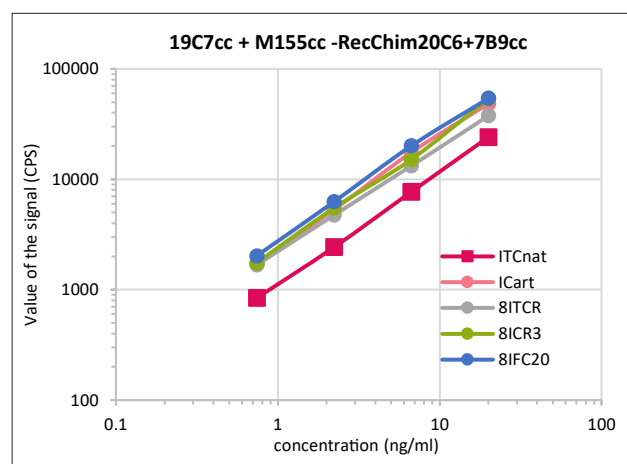


Figure 3. Immunoreactivity test of all developed recombinant troponin complexes and native analogue in two-step sandwich assays

- 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.