Troponin I

Porrima

Cardiac Troponin I (CLIA)

REF: PM107701 **Σ 100**

Intended use
The Porrima Troponin I assay is a Chemiluminescent Immunoassay (CLIA) for the quantitative determination of cardiac troponin I (cTnI) in **Human Serum or Plasma**. For professional use only.

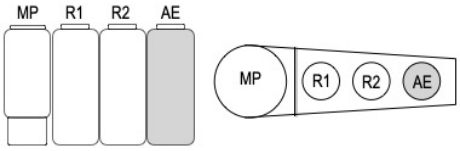
Summary
References[1-4]
Cardiac troponin I, often denoted as cTnI, is presented in cardiac muscle tissue by a single isoform with a molecular weight of 23.9 kDa. It consists of 209 amino acid residues. The theoretical pI of cTnI is 9.05. cTnI differs from other troponins due to its N-terminal extension of 26 amino acids. This extension contains two serines, residues 23 and 24, which are phosphorylated by protein kinase A in response to beta-adrenergic stimulation and important in increasing the inotropic response.
cTnI has been shown to be phosphorylated by protein kinase A, protein kinase C, protein kinase G, and p21-activated kinase 3.A significant part of cTnI released into the patient's blood stream is phosphorylated. For more than 15 years cTnI has been known as a reliable marker of cardiac muscle tissue injury. It is considered to be more sensitive and significantly more specific in diagnosis of the myocardial infarction than the "golden marker" of last decades – CK-MB, as well as total creatine kinase, myoglobin and lactate dehydrogenase isoenzymes.

Test principle
One-step Sandwich principle. Total duration of assay: **15 minutes**
In the first step, sample, paramagnetic microparticles coated with monoclonal anti-Troponin I antibody and monoclonal anti-Troponin I antibody-acridinium labeled conjugate are added into a reaction vessel. After incubation, Troponin I present in the sample binds to both anti-Troponin I antibody coated microparticle and anti-Troponin I antibody acridinium-labeled conjugate to form a sandwich complex. Microparticle is magnetically captured while other unbound substances are removed by washing.
In the second step, Pre-Trigger and Trigger Solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of Troponin I in the sample and the RLUs detected by the Porrima system.

Materials provided

MP	1 x 5.0 mL, anti-Troponin I antibody (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizers with preservative.
AE	1 x 5.0 mL, monoclonal anti-Troponin I antibody (mouse)-acridinium ester labeled conjugate in MES buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
C0	Calibrator, 1 vial, 1.0 mL, ready to use.
C1	Calibrator, 1 vial, 1.0 mL, ready to use.
C2	Calibrator, 1 vial, 1.0 mL, ready to use.

The position of each reagent component is shown in the figure below (front view on the left and top view on the right):



Materials required (but not provided)
- Porrima Chemiluminescent Immunoassay Analyzer
- CT2201 DiaSino ControlSet: 2 levels, L and H
- TR7701 Porrima TriggerPack: ①Pre-Trigger Solution, ②Trigger Solution
- WB7701 Porrima Wash Buffer (20X)
- RV7701 Porrima Reaction Vessels
- PW7701 Porrima Probe Wash Buffer
- SC7701 Porrima Sample Tubes (D13x75mm, D13x100mm or D16x100mm can be acceptable on Porrima system)

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/ face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Reagent handling
The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability
- Store at 2-8°C. Do not freeze.
- Store the Porrima reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.
- The Porrima Troponin I reagent kit can be stored onboard and used for a maximum of 28 days after opening at 2-8°C.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Centrifuge the specimens after clot formation is complete. Transfer the supernatants into tubes for storage or test within two hours after centrifugation.
- Specimens should be tested as soon as possible after sample collection. If testing is not completed within 8 hours, specimens should be tightly capped and refrigerated at 2-8°C. If testing will be delayed for more than 72 hours, specimens should be frozen at -20°C or below.
- Avoid repeated freeze and thaw cycles.

Assay procedure
- For optimal performance of this assay, operators should read the Porrima CLIA system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management, safety precaution, and maintenance. Prepare all required materials for the assay as well.
- Before loading the reagent kit on the machine for the first time, unopened reagent bottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage.
- Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended.
- If the microparticles cannot be resuspended, **DO NOT USE**. Contact DiaSino Technical Support for help. Do not invert opened reagent bottle.
- This assay requires 50 µL of sample for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration
The specific information of master calibration curve of Troponin I CLIA reagent kit is stored in the barcode attached in the reagent pack. It's used together with calibrators for the calibration of the specific reagent lot. When performing the calibration, scan the information of master calibration curve from the barcode into the system first, and then use the calibrators at two levels. Valid calibration curve is required before any Troponin I test. Recalibration is recommended every 28 days, or when a new reagent lot is used, or the quality controls are out of specified range. For detailed instruction of calibration, refer to the system operations manual.

Quality control
For quality control, use DiaSino ControlSet. In addition, other suitable control material can be used.

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Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Examine the assay system referring to the system operation manual. If the quality control results are still out of the specified range, please contact DiaSino Technical Support for help.

Calculation
The analyzer automatically calculates the analyte concentration of each sample.

Limitations - interference
- The results of Porrima Troponin I should be evaluated with all clinical and laboratory data available. If Troponin I test results do not agree with the clinical evaluation, additional tests should be performed.
- The false positive results may come from cross-reactions with some similar antibodies in blood, and similar epitopes from non-specific components in blood capturing fluorescent labeled antibodies.
- The false negative results may from some unknown substance blocking epitope adhering antibodies, unstable or degenerated Troponin I that cannot be identified due to prolonged time and temperature and storage condition of sample and reagent.
- Other factors may interfere with Porrima Troponin I and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
0.1-50 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.1 ng/mL. Values above the measuring range are reported as > 50 ng/mL

Lower detection limit
0.1 ng/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n = 21).

Expected values
0-0.3 ng/mL
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	0.24	0.018	7.66%	0.020	8.43%
Human Serum 2	3.49	0.228	6.54%	0.242	6.92%
Human Serum 3	37.28	1.495	4.01%	1.681	4.51%
ControlSet 1	5.46	0.343	6.29%	0.332	6.08%
ControlSet 2	19.58	0.854	4.36%	0.928	4.74%

Method comparison
A comparison of the Porrima Troponin I assay (y) with the Roche Elecsys Troponin I STAT assay (x) using 112 clinical samples gave the following correlations:
Linear regression
y = 1.004x + 0.024
r = 0.9725
The sample concentrations were between approx. 0 and 45 ng/mL.

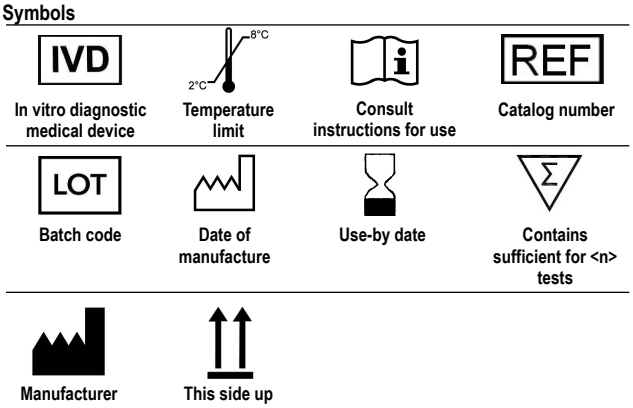
Analytical specificity
The Porrima Troponin I assay does not show any significant cross- reaction with the following substances (tested with troponin I concentrations of approximately 0.5 ng/mL and 3.5 ng/mL):
h-skeletal muscle troponin I 0.03 %, h-cardiac troponin T 0.05 %, h-skeletal muscle troponin T 0.0 % (not detectable) and human troponin C 0.0 % (not detectable).

Functional sensitivity
0.15 ng/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

1. Kozlowski, LP (21 October 2016). "IPC - Isoelectric Point Calculator". Biology Direct. 11 (1): 55. doi:10.1186/s13062-016-0159-9. PMC 5075173. PMID 27769290.
2. Solaro RJ, Moir AJ, Perry SV (1976). "Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart". Nature. 262 (5569): 615–616. doi:10.1038/262615a0. PMID 958429.
3. Layland J, Solaro RJ, Shah AM (2005). "Regulation of cardiac contractile function by troponin I phosphorylation". Cardiovascular Research. 66 (1): 12–21. doi:10.1016/j.cardiores.2004.12.022. PMID 15769444.
4. Labugger R, Organ L, Collier C, Atar D, Van Eyk JE (2000). "Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction". Circulation. 102 (11): 1221–1226. doi:10.1161/01.cir.102.11.1221. PMID 10982534.

Symbols



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