# DRG® Troponin I Elisa (cTnI) (EIA-2952)

CE

**Revised 18 Nov. 2010 rm (Vers. 5.1)** **USA: RUO**

***Please use only the valid version of the package insert provided with the kit.***

***This kit is intended for Research Use Only. Not for use in diagnostic procedures.***

Store at 2 to 8°C.

## Intended Use

The cTnI ELISA is intended for determination of cardiac troponin I in human serum.

## Principle of the Assay

The cTnI ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes four unique monoclonal antibodies directed against distinct antigenic determinants on the molecule. Three mouse monoclonal anti-troponin I antibodies are used for solid phase immobilization (on the microtiter wells). The fourth antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin I molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 90-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of tetramethylbenzidine (TMB) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of troponin I is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

## Reagents and Materials Provided

1. **Antibody-Coated Wells** (1 plate, 96 wells)
   Microtiter wells coated with mouse monoclonal anti-TnI.
2. **Reference Standard Set** (1 set, 1.0 ml/vial)
   Contains 0, 2.0, 7.5, 30, and 75 ng/ml TnI, lyophilized.
3. cTnI **Enzyme Conjugate** Reagent (13 ml/vial)
   Contains mouse monoclonal anti-TnI conjugated to horseradish peroxidase in Tris Buffer-BSA solution with preservatives.
4. **TMB Reagent** (11 ml/bottle)
   Contains one-step TMB solution.
5. **Stop Solution** (11 ml/bottle)
   Contains diluted hydrochloric acid (1N HCl).

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Materials Required But Not Provided

1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Cardiac Marker Tri-level Control; Cat. No. 685 (Bio-Rad Laboratories Diagnostics Group, Hercules, CA 94547)

Warnings and Precautions

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens32 or other appropriate national biohazard safety guidelines or regulations.33-34
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.

Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. ***The Reconstituted standards will be stable for up to 21 days when stored sealed at 2-8 °C.*** Discard the reconstituted Standards after 21 days. ***To assure long term (more than 21 days) maximum stability of the reconstituted Standards, they should be aliquoted and frozen (–20 °C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.***

3. Samples with expected Troponin I concentrations over 100 ng/ml may be quantitated by dilution with diluent available from vender.

Instrumentation

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

Specimen Collection and Preparation

1. The use of SERUM samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells *within 60 minutes* after collection.
3. Specimens which cannot be assayed within 24 hours of collection should be frozen at –20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

Procedural Notes

1. Pipetting Recommendations (single and multi-channel):
   Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Gently mix for 10 seconds.
4. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25°C) for 90 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)

9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
10. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
11. Incubate at room temperature for 20 minutes.
12. Stop the reaction by adding 100 µl of Stop Solution to each well.
13. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
14. Read absorbance at 450nm with a microtiter well reader ***within 15 minutes.***

Quality Control

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

Calculation of Results

1. Calculate the mean absorbance value ($OD_{450}$) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of troponin I (ng/ml) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Patient samples with cTnI concentrations greater than 75 ng/ml should be diluted 10-fold with vender's Troponin I Sample Diluent. The final cTnI values should be multiplied by 10 to obtain cTnI results in ng/ml.

Example of Standard Curve

Results of a typical standard run with absorbency readings at 450 nm shown on the Y axis against troponin I concentrations shown on the X axis.
**NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

**Example of Standard Curve**

| cTnI (ng/ml) | Absorbance (450 nm) |
|---|---|

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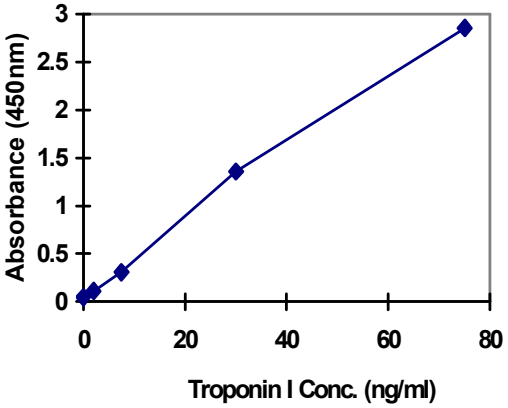
| | |
|---|---|
| 0 | 0.048 |
| 2.0 | 0.110 |
| 7.5 | 0.307 |
| 30 | 1.357 |
| 75 | 2.853 |

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