

HIGH SENSITIVITY RAT CARDIAC TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Cat. No. 2010-2-HSP

High Sensitivity ELISA For Determination of Cardiac Troponin-I in Rat Plasma

STORAGE CONDITIONS

On receipt store the lyophilized standard at or below minus 20°C. Store the remainder of the kit at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

EXPIRATION

The kit expiration date (six months from the date of shipment) is indicated on the package label

BACKGROUND

Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. The human troponin I subunit exists in three separate isoforms; two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnI) is about 40% dissimilar, has a molecular weight of 22,500 daltons, and has 31 additional amino acid residues that are not present on the skeletal isoforms. Antibodies made against the human cardiac isoform are immunologically different from antibodies made against the human skeletal isoforms, and the unique isoform and tissue specificity of cardiac troponin I is the basis for its use as an aid in the diagnosis of acute myocardial infarction (AMI) in humans.

PRINCIPLE OF THE ASSAY

The high sensitivity cTnI ELISA recognizes an epitope on rat cTnI that is relatively resistant to proteolysis in rat plasma, thereby improving detection capability. The assay uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horse radish peroxidase (HRP). The plasma sample is diluted with three volumes of plasma diluent and allowed to react simultaneously with the two antibodies, resulting in cTnI being sandwiched between the solid phase and HRP-conjugated antibodies. After one hour incubation at room temperature on a plate shaker, the wells are washed with wash solution to remove unbound HRP-conjugated antibodies. A solution of tetramethylbenzidine (TMB), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped by addition of 1N HCl changing the color to yellow. The concentration of cTnI is proportional to the absorbance at 450 nm.

REAGENTS AND MATERIALS PROVIDED

- Anti cTnI-Coated Wells (1 plate, 96 wells): Microtiter wells coated with cTnI antibodies
- cTnI Stock: Lyophilized rat cTnI (reconstitute with 0.40 ml H₂O)

- Standard Diluent (25 ml)
- Plasma Diluent (25 ml)
- 20X Wash Solution (50 ml)
- cTnI HRP Conjugate (11 ml): cTnI antibody conjugated to HRP
- TMB Reagent (11 ml): One-step TMB solution.
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Pipettes: P-10, P-200 & P-1000 or equivalent
3. Disposable pipette tips
4. Microtiter well reader capable of reading OD at 450 nm
5. Vortex mixer
6. Absorbent paper
7. Graph paper or appropriate PC graphing software
8. Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

1. 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.
2. Do not use reagents after expiration date and do not mix or use components from different kits.
3. Replace caps on reagents immediately. Do not switch caps.
4. Do not pipette reagents by mouth.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized cTnI stock by addition of 400 µl of de-ionized or distilled water. Mix gently several times over a period of 5-10 minutes. The concentration of cTnI in the reconstituted stock is indicated on the vial label.
3. Label 8 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0 ng/ml.
4. Into the tube labeled 10 ng/ml, pipette the volume of standard diluent detailed on the cTnI stock vial label. Then add the indicated volume of cTnI stock (shown on the cTnI stock vial label) and mix gently. This provides the 10 ng/ml standard.
5. Pipette 0.25 ml of standard diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0 ng/ml.
6. Prepare a 5 ng/ml standard by diluting and mixing 0.25 ml of the 10 ng/ml standard with 0.25 ml of diluent in the tube labeled 5 ng/ml. Similarly prepare the 2.5, 1.25, 0.625, 0.312 and 0.156 ng/ml standards by serial dilution.

NOTE: The reconstituted cTnI stock should be frozen immediately after use. It remains stable in frozen form for at

least 1 month at -20°C and 6 months at -70°C. Discard the working 10 – 0.156 ng/ml standards after use.

SAMPLE COLLECTION AND PREPARATION

Plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If plasma samples cannot be assayed within 4 hours of collection they should be frozen at -70°C and thawed only once prior to use.

We recommend that samples be assayed in duplicate. Prior to assay, plasma samples should be diluted four fold with plasma diluent. This can easily be accomplished by mixing 100 µl of each plasma sample with 300 µl of plasma diluent in a polypropylene micro centrifuge tube.

PROCEDURAL NOTES

- Standards and diluted plasma samples should be prepared immediately prior to use and used within 30 minutes.
- Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 10 minutes.
- It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 µl of cTnl HRP Conjugate into each well.**
- Dispense 100 µl of standards and diluted samples into appropriate wells.
- Thoroughly mix and incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture by flicking the plate contents into a waste container.
- Wash and empty the microtiter wells 6 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Dispense 100 µl of TMB Reagent into each well.
- Incubate at room temperature for 20 minutes on an orbital shaker at ~150 rpm.
- Stop the reaction by adding 100 µl of Stop Solution to each well.
- Gently mix. **It is important to make sure that all the blue color changes to yellow.**
- Read absorbance at 450 nm with a microtiter well reader within 5 minutes. **Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead.**
- If the absorbance values of the 4x diluted samples exceed those of highest standard, the 4x diluted plasma samples should be further diluted with standard diluent and re-tested (**do not use the plasma diluent for further dilution**).

CALCULATION OF RESULTS

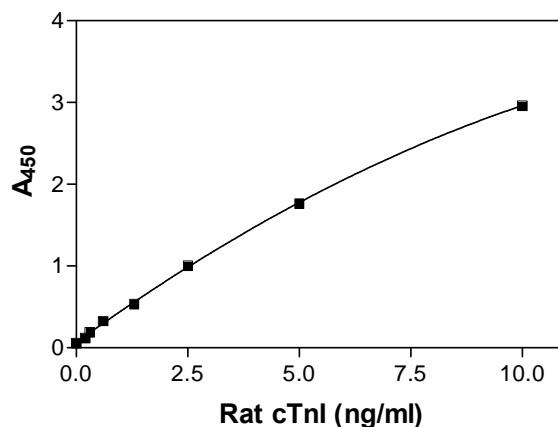
- Calculate the mean absorbance value (A_{450}) for the standards and samples.
- Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- Using the A_{450} values for each sample, determine the corresponding concentration of cTnl (ng/ml) from the standard curve. If using graphing software, we suggest using a point-to-point or a two site binding (hyperbola) fit of the data.
- Multiply the derived cTnl concentrations by the dilution factor (i.e., 4, if the recommended dilution was used) to obtain the actual plasma cTnl concentration.

EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against cTnl concentrations on the X axis are shown below.

NOTE: This standard curve is for the purpose of illustration only.

cTnl (ng/ml)	Absorbance (450 nm)
10.000	2.965
5.000	1.766
2.500	1.004
1.250	0.539
0.625	0.329
0.313	0.193
0.156	0.119
0	0.054



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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