

ULTRA-SENSITIVE MOUSE CARDIAC TROPONIN-I ELISA

Life Diagnostics, Inc., Cat. No. CTNI-1-US

ULTRA-SENSITIVE ELISA FOR MEASUREMENT OF MOUSE CARDIAC TROPONIN-I

STORAGE

When the kit is received, store the lyophilized standard at or below minus 20°C. Store the remainder of the kit in the refrigerator at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant. The kit expiration date is indicated on the package.

BACKGROUND

Troponin is the contractile regulating protein complex of striated muscle. It consists of three subunits: troponin I, T, and C. Troponin-I exists in three isoforms; one in fast-twitch skeletal muscle, one in slow-twitch skeletal muscle, and one in cardiac muscle. After muscle injury by trauma or ischemia, troponin is released into the bloodstream. The sequence of cardiac troponin-I (cTnI) is significantly different from the skeletal muscle isoforms, thus allowing generation of specific anti-cTnI antibodies and development of cTnI specific immunoassays. Because cTnI is uniquely expressed in the heart it provides a specific biomarker of cardiac damage.

PRINCIPLE OF THE ASSAY

The ultra-sensitive cTnI ELISA uses two affinity purified antibodies. A rabbit anti-cTnI polyclonal antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-cTnI peptide-specific polyclonal antibody is conjugated to horseradish peroxidase (HRP) and used for detection. Samples (serum or plasma) and standards (200 µl) are pipetted into the microtiter wells and incubated for 2 hours on a plate shaker. After washing the wells, 100 µl of diluent and 100 µl of HRP-conjugated anti-cTnI are pipetted into each of the microtiter wells. The plate is incubated for one hour on a plate shaker. During this step, cTnI becomes sandwiched between the solid phase and HRP-conjugated antibodies. The wells are then washed to remove unbound HRP-conjugated antibodies. TMB, an HRP substrate (100 µl), 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 (100 µl), changing the color to yellow, and absorbance at 450 nm is measured. The concentration of cTnI is proportional to the absorbance at 450 nm and is derived from a standard curve.

REAGENTS AND MATERIALS PROVIDED

- Anti cTnI-coated microtiter plate (96 wells 12 x 8-well strips)
- cTnI Stock: Lyophilized mouse cTnI
- cTnI Diluent (25 ml)
- cTnI HRP Conjugate (11 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (11 ml): HRP substrate solution
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes & tips: P-10, P-200 & P-1000 or equivalent
- Plate shaker

- Plate washer
- Plate reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

- Avoid contact with 1N HCl (Stop Solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.

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

Sufficient reagents are provided for the preparation of at least two standard curves.

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized cTnI stock by adding 200 µl of deionized or distilled water. Mix gently several times over a period of 5 minutes.
3. Label 7 polypropylene tubes as 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 ng/ml.
4. Into the tube labeled 2.5 ng/ml, pipette the volume of cTnI 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 2.5 ng/ml standard.
5. Pipette 0.50 ml of cTnI diluent into the tubes labeled 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 ng/ml.
6. Prepare a 1.25 ng/ml standard by diluting and mixing 0.50 ml of the 2.5 ng/ml standard with 0.50 ml of diluent in the tube labeled as 1.25 ng/ml. Similarly prepare the 0.625, 0.312, 0.156, 0.078, and 0.039 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 standards after use.

SAMPLE COLLECTION AND PREPARATION

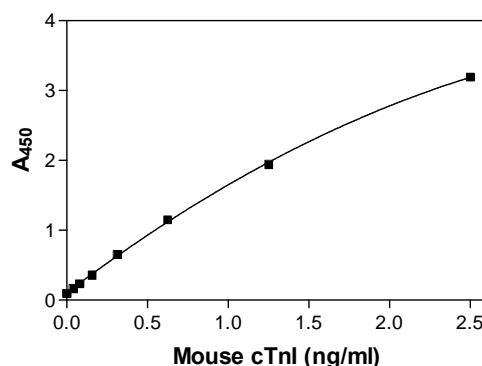
Serum or plasma should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same). If samples cannot be assayed immediately they should be frozen at -70°C and thawed only once prior to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

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.

2. Dispense 200 μ l of standards and samples into appropriate wells.^a
3. Incubate on an orbital shaker (150 rpm) at 25°C for 2 hours.^b
4. Using a plate washer, wash the microtiter wells 5 times with 1x wash solution (400 μ l per well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ l of cTnl diluent to each well.
7. Add 100 μ l of HRP conjugate to each well.
8. Incubate on an orbital shaker (150 rpm) at 25°C for 1 hour.
9. Wash the plate as described in steps 4 and 5.
10. Dispense 100 μ l of TMB Reagent into each well.
11. Incubate on an orbital shaker (150 rpm) at 25°C for 20 minutes.
12. Stop the reaction by adding 100 μ l of Stop Solution to each well.
13. Gently mix until all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.
15. If absorbance values exceed the high standard, the samples should be appropriately diluted with cTnl diluent and re-determined.

cTnl (ng/ml)	A ₄₅₀
2.5	3.194
1.25	1.944
0.625	1.147
0.313	0.658
0.156	0.359
0.078	0.236
0.039	0.165



CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A₄₅₀) for the standards and samples.
2. Construct a standard curve by plotting the A₄₅₀ 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.
3. Using the A₄₅₀ values for each sample, determine the corresponding concentration of cTnl (ng/ml) from the standard curve.
4. If available, graphing software should be used to analyze the data. Depending on the range of the standard curve used for analysis, good fits may be obtained with linear regression analysis or by fitting the data to a polynomial second order equation. We recommend the latter option.

REPRESENTATIVE STANDARD CURVE

Results of a typical standard curve with A₄₅₀ 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.

PROCEDURAL NOTES

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. Standards should be used within 30 minutes of preparation.
3. Pipetting of all reagents into the microtiter plate should be completed within 5 minutes at each step (we strongly recommend the use of multipipettors).
4. Standards and samples should be run in duplicate.

REFERENCES

1. Hou G, Dick R, Abrams GD, Brewer GJ. Tetrathiomolybdate protects against cardiac damage by doxorubicin in mice. *J Lab Clin Med.* 146(5):299-303 (2005)
2. Tao L, Liu H-R, Gao F, Qu Y, Christopher TA, Lopez BL and Ma XL. Mechanical traumatic injury without circulatory shock causes cardiomyocyte apoptosis: role of reactive nitrogen and reactive oxygen species. *Am J Heart Circ Physiol.* 288:H2811-H2818 (2005)
3. Haas MS, Alicot EM, Schuerpf , Chiu I, Li J, Moore FD and Carroll MC. Blockade of self-reactive IgM significantly reduces injury in a murine model of acute myocardial infarction. *Cardiovascular Research.* 87:618-627 (2010)

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For technical assistance please email us at
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^a 100 μ l of sample may be used if volume is limiting. However, the volume of all other samples and standards used in the assay must also be 100 μ l. Absorbance values will be slightly lower.

^b The ELISA was validated at Life Diagnostics using temperature controlled shaking incubators at 25°C. Assays performed at lower or higher temperatures may result in lower or higher absorbance values.