

HIGH SENSITIVITY BOVINE CARDIAC TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Catalog No. CTNI-11-HS

HIGH SENSITIVITY ELISA FOR DETERMINATION OF CARDIAC TROPONIN-I IN BOVINE SERUM

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 troponin I subunit exists in three isoforms: one in fast-twitch skeletal muscle, one in slow-twitch skeletal muscle, and one in cardiac muscle. At the sequence level, cardiac troponin-I (cTnI) is significantly different from the skeletal isoforms, and antibodies can be prepared that specifically recognize cTnI. The unique isoform and tissue specificity of cTnI are the basis for its use as a marker of cardiac muscle damage.

PRINCIPLE OF THE ASSAY

The ELISA uses two different affinity purified cTnI specific antibodies that have been used with great success since 2006 in our mouse and rat cTnI ELISA kits.¹ The assay uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horseradish peroxidase (HRP). The serum sample is allowed to react simultaneously with the two antibodies, resulting in cTnI being sandwiched between the solid phase and HRP-conjugated antibodies. After incubation for one hour at room temperature on a plate shaker, the wells are washed 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)
- cTnI Stock: Lyophilized bovine cTnI (reconstitute with 0.40 ml H₂O)
- cTnI Diluent (12 ml)
- cTnI HRP Conjugate (11 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (11 ml): HRP substrate solution
- Stop Solution (11 ml): 1N HCl

¹ A list of literature references citing use of our cTnI ELISA kits may be found on our website on the "Cardiac Biomarker ELISA's" page.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Micro-plate incubator/shaker with mixing speed of ~150 rpm
- 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.
- 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 deionized 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 7 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/ml.
4. Into the tube labeled 10 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 10 ng/ml standard.
5. Pipette 250 µl of cTnI diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/ml.
6. Prepare a 5 ng/ml standard by diluting and mixing 250 µl of the 10 ng/ml standard with 250 µl ml of diluent in the tube labeled as 5 ng/ml. Similarly prepare the 2.5, 1.25, 0.625, 0.313 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

Serum 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 serum 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 100 μl of cTnl HRP Conjugate into each well.**
3. Dispense 100 μl of standards and samples into appropriate wells.
4. Thoroughly mix for 10-15 seconds. It is very important to mix completely.
5. Incubate on an orbital shaker (150 rpm) at room temperature ($18-25^{\circ}\text{C}$) for 60 minutes.
6. Remove the incubation mixture using a plate washer or by flicking the plate contents into a bio-waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μl /well) or a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 μl of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate on an orbital shaker (150 rpm) at room temperature for 20 minutes.
11. Stop the reaction by adding 100 μl of Stop Solution to each well.
12. Gently mix until all the blue color changes to yellow.
13. Read absorbance at 450 nm with a plate reader within 15 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.***
14. If absorbance values exceed the high standard, the samples should be appropriately diluted with sample diluent (catalog number 2010-HSD, Life Diagnostics, Inc.) and re-tested. Samples with absorbance values below those of the 0.156 ng/ml standard should be assigned a zero troponin-I value.

CALCULATION OF RESULTS

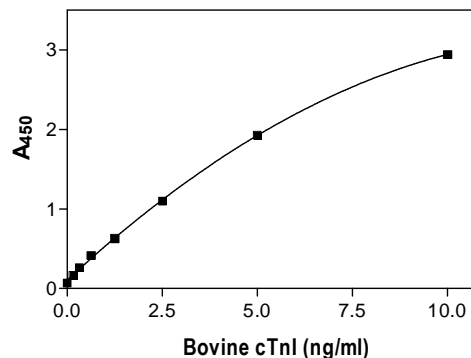
1. Calculate the mean absorbance value (A_{450}) for each of the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} 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. We find that a good fit of the data may be obtained with a second order polynomial or a two-site binding model. Alternatively, standard curves may be generated using a point-to-point fit.

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)	A_{450}
10	2.945
5	1.926
2.5	1.100
1.25	0.628
0.625	0.415
0.313	0.260
0.156	0.164



LIMITATIONS OF THE PROCEDURE

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. Plasma cannot be used with this kit.

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For technical assistance please email us at techsupport@lifediagnosics.com