

HIGH SENSITIVITY GUINEA PIG CARDIAC TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Cat. No. 2010-7-HS

HIGH SENSITIVITY ELISA FOR DETERMINATION OF CARDIAC TROPONIN-I IN GUINEA PIG 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; two in fast-twitch and slow-twitch skeletal muscle fibers, 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 high sensitivity cTnI ELISA recognizes an epitope on guinea pig cTnI that is relatively resistant to proteolysis in guinea pig serum, 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 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 guinea pig cTnI (reconstitute with 0.20 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

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- 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

- Serum must be collected using methods that do not cause damage to the heart. **Do not collect serum by cardiac puncture.**
- 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

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 addition of 200 μ l 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 7 polypropylene tubes as 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0.20 ng/ml.
4. Into the tube labeled 12.5 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 12.5 ng/ml standard.
5. Pipette 0.25 ml of cTnI diluent into the tubes labeled 6.25, 3.13, 1.56, 0.78, 0.39, and 0.20 ng/ml.
6. Prepare a 6.25 ng/ml standard by diluting and mixing 0.25 ml of the 12.5 ng/ml standard with 0.25 ml of diluent in the tube labeled as 5 ng/ml. Similarly prepare the 3.13, 1.56, 0.78, 0.39, and 0.20 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 12.5 – 0.2 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. **Do not collect serum by cardiac puncture.**

PROCEDURAL NOTES

- Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
- Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 5 minutes.
- We recommend that standards and samples be run in duplicate.
- 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 cTnI HRP Conjugate into each well.**
- Dispense 100 µl of standards and samples into appropriate wells.
- Incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture using a plate washer or by flicking the plate contents into a bio-waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution using a plate washer (400 µl/well). The wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
- Incubate on an orbital shaker (150 rpm) at room temperature for 20 minutes
- Stop the reaction by adding 100 µl of Stop Solution to each well.
- Gently mix until all the blue color changes to yellow.
- Read absorbance at 450 nm with a plate 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 absorbance values exceed the high standard, the samples should be appropriately diluted with cTnI diluent (catalog number 2010-HSD, Life Diagnostics, Inc.) and re-determined. Samples with absorbance values below those of the 0.156 ng/ml standard should be assigned a zero troponin-I value.

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 cTnI (ng/ml) from the standard curve.
- If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to-point fit.

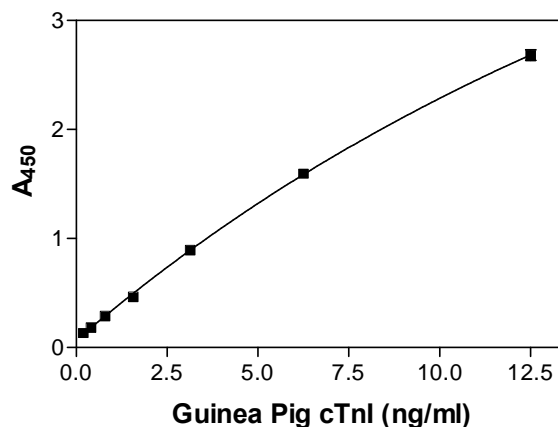
EXAMPLE OF STANDARD CURVE

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

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

cTnI (ng/ml)	Absorbance (450 nm)
12.5	2.68
6.25	1.60
3.13	0.89
1.56	0.47
0.78	0.29
0.39	0.18
0.20	0.13

Representative Guinea Pig cTnI Standard Curve



LIMITATIONS OF THE PROCEDURE

- 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.
- Plasma cannot be used with this kit.

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