DATA SHEET

Rat Cardiac Troponin-I ELISA

For the quantitative determination of cardiac troponin-I in rat plasma.

Cat. No. KT-480

For Research Use Only.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Rat Cardiac Troponin-I ELISA is an enzyme immunoassay for the quantitative determination of cardiac troponin-I in rat plasma. For research use only.

INTRODUCTION
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 study of acute myocardial infarction (AMI) in humans.

PRINCIPLE
The high sensitivity **K-*ASSAY*®** Rat Cardiac Troponin-I 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 horseradish 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 to remove unbound HRP-conjugated antibodies. A solution of TMB (Tetramethylbenzidine), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of cTnI is proportional to the absorbance at 450 nm.

COMPONENTS
- Anti-cTnI-coated microtiter wells, 96 wells
- Rat cTnI Calibrator (lyophilized), reconstitute with 0.40 mL H_2O
- Calibrator Diluent, 25 mL
- Plasma Diluent, 25 mL
- cTnI HRP Conjugate, 11 mL
- Wash Solution (20X), 50 mL
- TMB Reagent, 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 mL)
- Microtiter plate reader capable of reading OD at 450 nm.

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 de-ionized water.

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CALIBRATOR 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 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0 ng/mL.
4. Into the tube labeled 5 ng/mL, pipette the volume of calibrator diluent detailed on the cTnI calibrator vial label. Then add the indicated volume of cTnI calibrator (shown on vial label) and mix gently. This provides the 5 ng/mL calibrator.
5. Pipette 0.25 mL of calibrator diluent into the tubes labeled 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0 ng/mL.
6. Prepare a 2.5 ng/mL calibrator by diluting and mixing 0.25 mL of the 5 ng/mL calibrator with 0.25 mL of diluent in the tube labeled as 2.5 ng/mL. Similarly prepare the 1.25, 0.625, 0.312, 0.156 and 0.078 ng/mL calibrators by serial dilution.

Note: The reconstituted cTnI calibrator 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 5 – 0.078 ng/mL calibrators 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

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

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of cTnI HRP Conjugate into each well.
3. Dispense 100 µL of calibrators and diluted samples into the appropriate wells.
4. Thoroughly mix and incubate at room temperature (18-25°C) on a plate shaker (150 rpm) for one hour.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. 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.
7. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
8. Dispense 100 µL of TMB Reagent solution into each well.
9. Incubate on a plate shaker (~150 rpm) at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µL of Stop Solution to each well.
11. Gently mix. It is important to make sure that all the blue color changes to yellow.
12. Read absorbance at 450 nm with a microtiter plate reader *within 5 minutes.* **Please note: Due to plate reader differences, the high calibrator absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead.**
13. If the absorbance values of the 4x diluted samples exceed those of highest calibrator, the 4x diluted plasma samples should be further diluted with calibrator diluent and re-tested (Do not use the plasma diluent for further dilution).

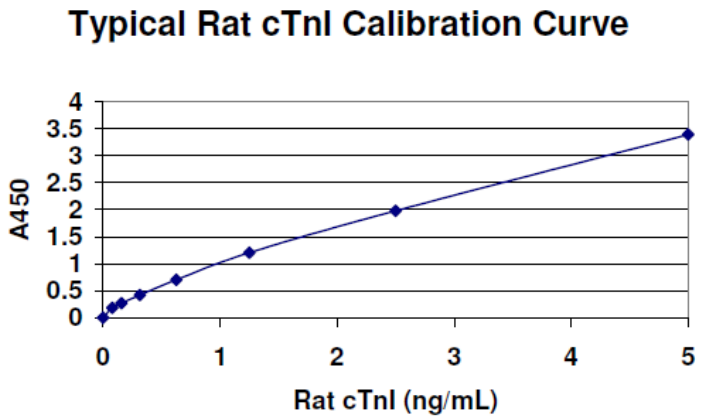
CALCULATION OF RESULTS

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

TYPICAL CALIBRATION CURVE

Results of a typical calibration run with optical density reading at 450 nm shown on the Y axis against cTnI concentration shown on the X axis are illustrated below. This calibration curve is for the illustration purpose only and should not be used to calculate unknowns. A calibration curve should be run for each assay.

cTnI (ng/mL)	Absorbance (450 nm)
5	3.395
2.5	1.980
1.25	1.203
0.625	0.704
0.313	0.421
0.156	0.268
0.078	0.182
0	0



STORAGE

Store kit at 4℃. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.

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 kits with different lot numbers.
3. Do not pipette reagents by mouth.
4. Replace caps on reagents immediately. Do not switch caps.

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 package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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