

ELISA kits available from ADI:

Catalog# ProdDescription

600-400-CTN Dog Cardiac Troponin 1 (Tn-I) ELISA Kit
600-420-CTN Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit
600-430-MTN Monkey Skeletal Muscle Troponin 1 (Tn-I) ELISA
600-440-CTN Mouse Cardiac Tn-I ELISA kit for plasma samples
600-450-CTN Mouse Cardiac Tn-I ELISA kit for serum samples
600-460-MTN Mouse Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-470-CTN Pig Cardiac Troponin 1 (Tn-I) ELISA Kit
600-480-CTN Rabbit Cardiac Troponin 1 (Tn-I) ELISA Kit
600-510-MTN Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-600-DMY Dog Myoglobin ELISA Kit
600-610-HMY Human Myoglobin ELISA Kit
600-620-MMY Monkey Myoglobin ELISA Kit
600-630-MMY Mouse Myoglobin ELISA Kit
600-640-PMY Pig Myoglobin ELISA Kit
600-650-RMY Rabbit Myoglobin ELISA Kit
600-660-RMY Rat Myoglobin ELISA Kit

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin **Turkey:** IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM

Cat: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit:** CRP, IgG

See Details at the web site or Contact ADI

Instruction Manual No. M-600410-CTN

Human Cardiac Troponin-I (cTnI)

ELISA KIT Cat. # 600-410-CTN

For Quantitative Determination of Cardiac Troponin-I (cTnI) in Human Serum



India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400

Fax: +91-11-42208444

Email: customerservice@atzlabs.com

Web: www.atzlabs.com



DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

Human Cardiac Troponin-I (cTnI) ELISA KIT
Cat. No. 600-410-CTN

Kit Components, 96 tests	Cat #
Anti-Human Troponin-I (cTnI) coated strip plate (8 wells x 12 strips), #600410-1	1 plate
Human cTnI Reference Standard set contains 0, 2.0, 7.5, 30 and 75 ng/ml, lyophilized. <i>Reconstitute with dH₂O volume specified on the vial</i> , #600410-2A-E	5 vials
Anti-Human TnI-HRP Conjugate, 13 ml, #600410-3	1 bottle
TMB Substrate, 11 ml, #600410-TMB	1 bottle
Stop solution, 11 ml, #600410-SS	1 bottle
Instruction Manual, # M-600410	1 manual

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 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 iso form and tissue specificity of cTnI are the basis for its use as a marker of cardiac muscle damage.

Recent literature describes cardiac troponin (I or T) as the preferred biomarker for myocardial damage. The cardiac troponins exhibit myocardial tissue specificity and high sensitivity. Cardiac TnI and CK-MB have similar release patterns (4-6 hours after onset of pain) but the level of cTnI remains elevated for a much longer period of time (6-10 days).

ADI's Human troponin-I ELISA provides is a rapid, specific and sensitive assay for measuring Human cardiac troponin-I in serum. This kit is not recommended for plasma samples.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate TROPONIN-I concentrations. Read off the TROPONIN-I concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:20K then the values must be multiplied by 20,000 and results are expressed as ug/ml.

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.

PERFORMANCE CHARACTERISTICS

Detection Limit: The minimum cTnI concentration detectable using this assay is below 2.0 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

Specificity: The antibodies used in this kit are specific for Human Troponin-I and have shown no cross-reactivity with other troponins or proteins.

Species Crossreactivity: No cross reactivity was observed for the following components at concentrations up to the levels indicated:

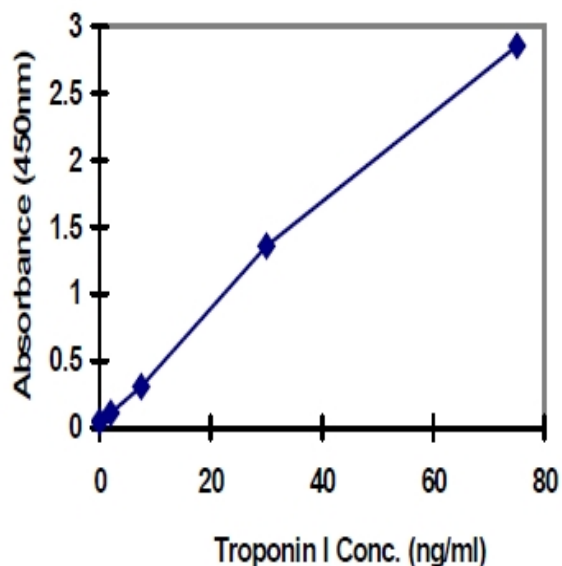
Material Tested	Test Concentration
Rabbit skeletal muscle troponin C	2,500 ng/ml
Human Cardiac troponin C	2,500 ng/ml
Human skeletal muscle troponin I & T	2,500 ng/ml
Hemoglobin	1.2 g/dl
Bilirubin	20 mg/dl
Cholesterol	500 mg/dl
Triglyceride	1,000 mg/dl
Total Protein	10 g/dl

Species crossreactivity with cTnI from mouse, rat etc have not been studied.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450 nm}	Calculated Conc _n
A1, A2	Standard A 0 ng/ml	0.048	
B1, B2	Standard B 2.0 ng/ml	0.110	
C1, C2	Standard C 7.5 ng/ml	0.307	
D1, D2	Standard D 30.0 ng/ml	1.357	
E1, E2	Standard E 75.0 ng/ml	2.853	
F1, F2	Sample 1	1.318	27.5 ng/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Human cardiac Troponin-I (cTnI) ELISA kit is based on binding four mouse monoclonal antibodies directed against unique antigenic determinants of Human cTnI molecule. Three of these antibodies are immobilized on the microtiter well plates, and the other is conjugated to the enzyme horseradish peroxidase. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin 1 molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of Troponin-I present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm, and the concentration of Troponin-I in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Human Troponin-I ELISA Kit is for research use only.

Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HbsAg, HIV1/2 and HCV by FDA-approved methods. However, all human blood samples should be considered potentially infectious and appropriate precautions should be taken in handling these specimens.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture; allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. Avoid grossly hemolytic, lipemic or turbid samples. **Cell or tissues extract samples have not been optimized.**

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

DILUTION OF SAMPLES

Samples containing more than 75.0 ng/ml Human cTnI should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

We do not recommend using PLASMA with the kit.

REAGENT PREPARATION & TEST PROCEDURE

(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Label or mark the microtiter well strips to be used on the plate.

1. Reconstitute the lyophilized Reference Standards with 1.0 ml of distilled water or volume specified on the vial label and other dilution instructions provided on the vial. Mix gently for 5-10 min at room temp. Store unused Reference Standards in aliquots at -70°C. They are stable for at least 1 month at -20°C and 6 months at -70°C. Do not freeze and thaw the standards.
2. Samples with expected troponin concentration over 100 ng/ml may be quantitated by diluting in a diluent available from ADI.
3. Pipet **100 ul standards and diluted samples** into appropriate wells. Pipette **100 ul of anti-cTnI-HRP conjugate** into each well. Mix gently for 5-10 seconds, and incubate at room temperature (20-25°C) for **90 minutes**.
4. Remove or aspirate the plate contents and **wash the wells 4-5 times** with 300 ul of distilled or deionized water using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

5. **Add 100 ul of TMB Substrate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temperature. Blue color develops. This step can be reduced or increased by \pm 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.
6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently. Blue color turns yellow.
7. Measure the **absorbance at 450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.
8. 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 and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero troponin-I value

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.