ELISA kits available from ADI:

Catalog#	ProdDescription
600-410-CTN	Human 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-600400-CTN

Dog Cardiac Troponin-I (cTnI)

ELISA KIT Cat. # 600-400-CTN

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

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Dog Cardiac Troponin-I (cTnI) ELISA KIT Cat. No. 600-400-CTN

Kit Components, 96 tests	Cat #
Anti-Dog Troponin-I coated strip plate (8 wells x 12 strips)	600400-1
Dog Troponin-I Reference Standard, lyophilized, *Reconstitute with 0.4 ml dH_20 (or volume specified on the vial). Lot specific stock concentration is indicated on the vial*	600400-2
Anti-Dog Troponin-I-HRP Conjugate, 11 ml	600400-3
cTnI-Diluent, 12 ml	600400-4
Wash Buffer (20x), 50 ml	600400-WB
TMB Substrate, 11 ml	600400-TMB
Stop solution, 11 ml	600400-SS
Instruction Manual	M-600400

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.

ADI's dog troponin-I ELISA provides is a rapid, specific and sensitive assay for measuring dog troponin-I in serum or other biological fluids.

DILUTION OF SAMPLES

Samples containing more than 10.0 ng/ml TROPONIN-I should be further diluted with cTnI diluent and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

We do not recommend using PLASMA with the kit.

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 TROPONIN-I concentration detectable using this assay is below 0.1 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.

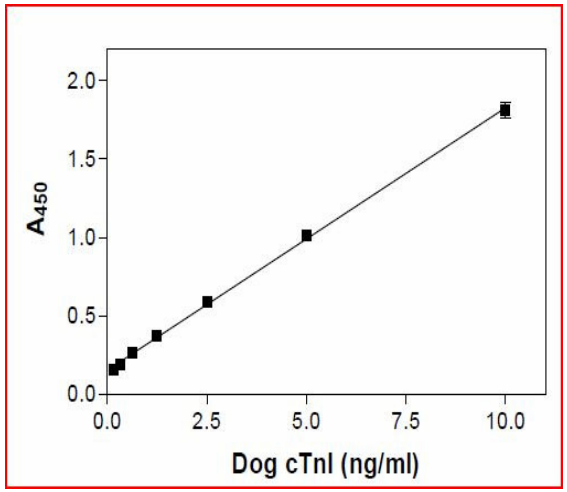
Specificity: The antibodies used in this kit are specific for Dog Troponin-I and have shown no cross-reactivity with other troponins or proteins.

Species Crossreactivity: Cross-reactivity of Dog Troponin-I ELISA kit with other animals has not been tested. ADI has Troponin-I ELISA kits for mouse, rat, human, pig, monkey, rabbit and g. pig.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Neg. Control** 0 ng/ml	0.086	
B1, B2	**Standard A** 0.156 ng/ml	0.16	
C1, C2	**Standard B** 0.313 ng/ml	0.19	
D1, D2	**Standard C** 0.625 ng/ml	0.27	
E1, E2	**Standard D** 1.25 ng/ml	0.38	
F1, F2	**Standard E** 2.5 ng/ml	0.59	
F1, F2	**Standard F** 5.0 ng/ml	1.01	
F1, F2	**Standard G** 10.0 ng/ml	1.81	
G1, G2	**Sample 1**	1.09	5.22 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

Dog Troponin-I ELISA kit is based on binding of Dog Troponin-I from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. 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 Dog Troponin-I ELISA Kit is for research use only.

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. **Plasma is not compatible in this kit. Cell or tissues extract samples have not been optimized.**

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with distilled or deionized water to 950 ml water (total volume 1000 ml). Store at room temperature for the entire use of the kit.

2. Standard preparation-it is provided as lyophilized stock. See detailed preparation on page 3.

STORAGE AND STABILITY

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

TEST PROCEDURE

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

1. Reconstitute the lyophilized Reference Standard with 0.4 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. **The stock concentration will be 10.0 ng/ml.** Store unused Reference Standard in aliquots at -20°C. Do not freeze and thaw the standard.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 10.0, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 ng/ml.

3. For standard G (10.0 ng/ml) pipette the volume of cTnI diluent and add the indicated volume of cTn1 stock (as described on the cTn1 stock vial label) and mix gently. Prepare the remaining standards as shown below.

Dog Troponin-I Stds	Stock Volume	cTnI diluent	Final Volume
Std G (10.0 ng/ml)	500 ul	0	500 uL
Std F (5.0 ng/ml)	250 ul of Std G	250 uL	500 uL
Std E (2.5 ng/ml)	250 ul of Std F	250 uL	500 uL
Std D (1.25 ng/ml)	250 ul of Std E	250 uL	500 uL
Std C (0.625 ng/ml)	250 ul of Std D	250 uL	500 uL
Std B (0.313 ng/ml)	250 ul of Std C	250 uL	500 uL
Std A (0.156 ng/ml)	250 ul of Std B	250 uL	500 uL
Negative (0 ng/ml)	0	250 uL	250 uL

Notes: When preparing the serial dilutions of the standards, gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme, you will have 250 ul of all standards (B-F) and 500 ul of Std. A. You would need 200 ul of each standard (100 ul in duplicate).

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

4. Pipette **100 ul of anti-cTnI-HRP conjugate** into each well.

5. Pipet **100 ul standards and diluted samples** into appropriate wells. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes on an orbital shaker (100-150 rpm)**. If an automated shaker is not available, the plate can be mixed manually every few minutes.

6. Remove or aspirate the plate contents and **wash the wells 4-5 times** with 300 ul of 1x wash buffer 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.

7. **Add 100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature **on an orbital shaker (100-150 rpm)**. Blue color develops. This step can be reduced or increased by ± 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.

8. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently. Blue color turns yellow.

9. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 minutes after stopping.

10. 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^{o}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.