Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	91	103
	Range (%)	84-101	94-118
1:4	Average% of Expected	92	101
	Range (%)	88-102	98-106

REFERENCES

1. Townsend PJ, et al. (1994). Genomics 21 (2): 311–6.

2. Zong, N. C. et al. (2013). Circulation Research 113 (9): 1043–53.

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4. Revera M, et al. (2007). Cardiovascular Journal of Africa 18 (3): 146–53.

5. Fujino N, et al. (2002). The American Journal of Cardiology 89 (1):

29–33.

Mouse CTNT Immunoassay

Catalog Number: SEKM-0150

For the quantitative determination of Mouse CTNT concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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BMP2

Factors assayed for cross-reactivity

Recombinant mouse	Recombinant rat	Recombinant human
	BMP1	
	BMP2	
	BMP3	
	BMP4	
	IL-2	
	IL-4	
	IFN-γ	
	TGF-β1	
	TLR1	
	TNF-α	

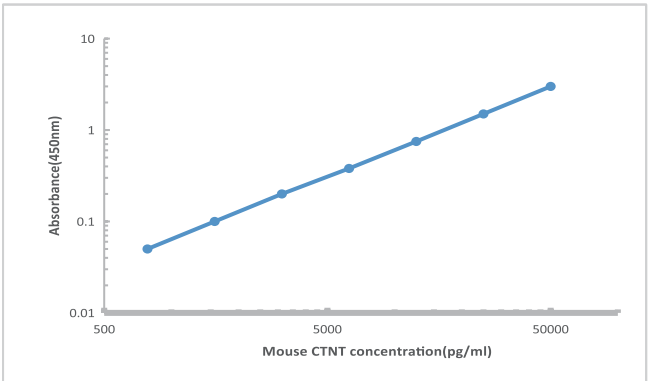
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of CTNT spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CTNT in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	95	87-104
Cell culture supernatants	96	89-108

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CTNT in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.



Representative standard curve for CTNT ELISA.

Performance Characteristics

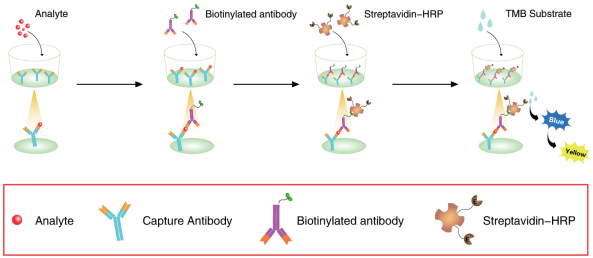
SENSITIVITY: The minimum detectable dose was 400 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant mouse CTNT The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

BACKGROUND

Cardiac Troponin T (TnT),is a protein which in humans is encoded by the TNNT2 gene.Cardiac TnT is the tropomyosin-binding subunit of the troponin complex,which is located on the thin filament of striated muscles and regulates muscle contraction in response to alterations in intracellular calcium ion concentration.As part of the Troponin complex,the function of cTnT is to regulate muscle contraction.Mutations of this gene may be associated with mild or absent hypertrophy and predominant restrictive disease,with a high risk of sudden cardiac death.Advancement to dilated cardiomyopathy may be more rapid in patients with TNNT2 mutations than in those with myosin heavy chain mutations.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CTNT has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTNT present is captured by the coated antibody after incubation.Following extensive washing, a biotin-conjugate antibody specific for CTNT is added to detect the captured CTNT protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of Samples.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the CTNT concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CTNT ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.040	0.044	0.042	-
781.25	0.156	0.148	0.152	0.110
1562.5	0.228	0.215	0.221	0.179
3125	0.338	0.320	0.329	0.287
6250	0.537	0.508	0.523	0.480
12500	0.873	0.825	0.849	0.807
25000	1.421	1.344	1.382	1.340
50000	2.315	2.189	2.252	2.210

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

⇩

Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-Mouse CTNT antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature (25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-25 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,50000 pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

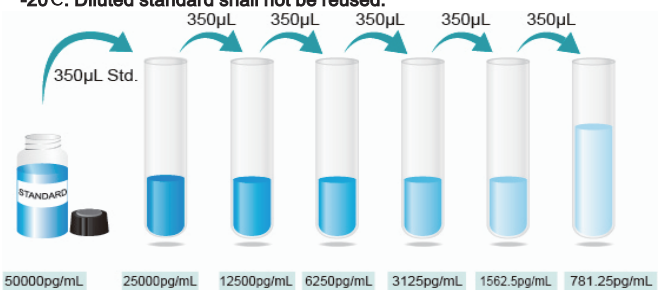
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning:** Bring all kit components and Sample to room temperature (20-25℃) before use.
2. **Wash Buffer-** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample -** Reconstitute the Standard with 0.7mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 50000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 350uL of Standard/Sample Diluent into 25000pg/ml tube and the remaining tubes. Use the stock solution of 50000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 50000pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0pg/mL).***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**



Preparation of CTNT standard dilutions

4. **Working solution of Biotin-Conjugate anti-mouse CTNT antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**