

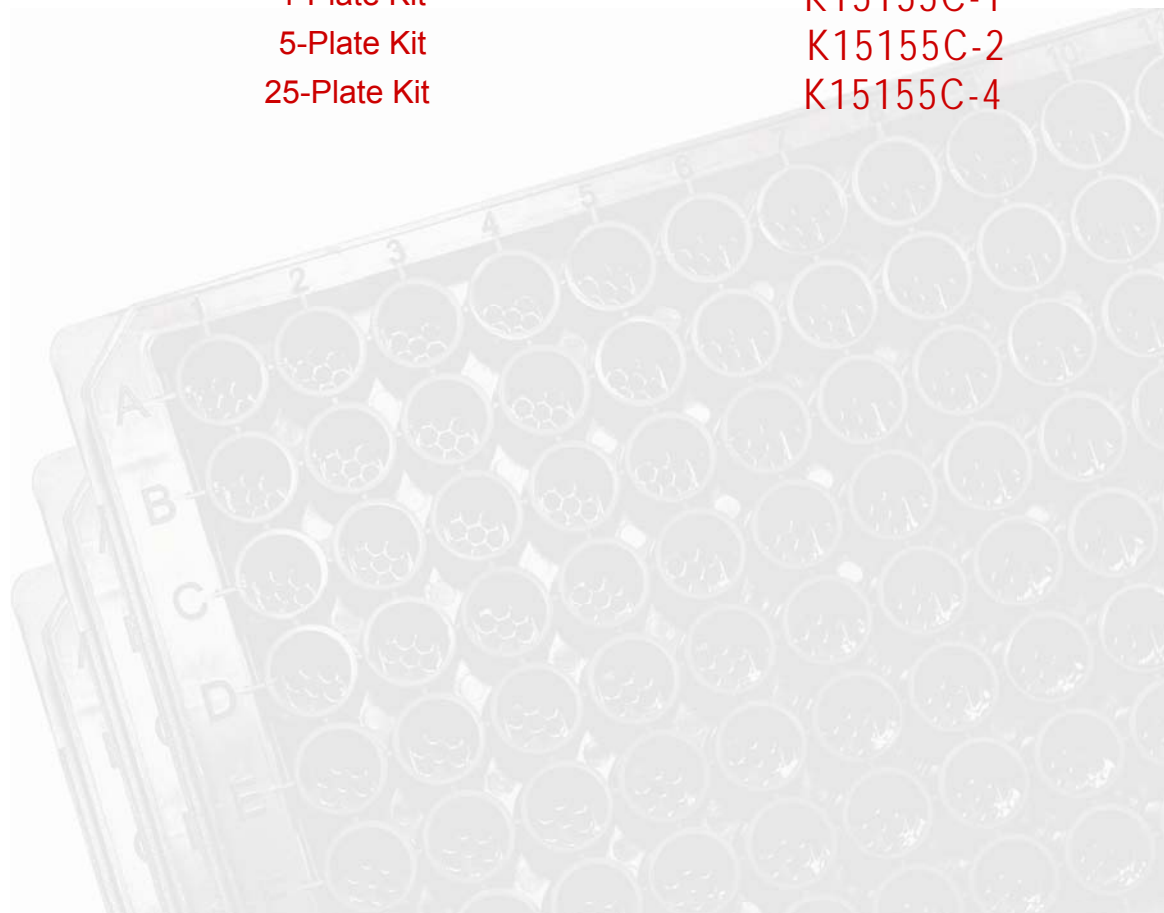
Meso Scale Discovery[®]

MULTI-SPOT[®] Assay System

Cardiac Injury Panel 2 (rat) Assay Kit

1-Plate Kit
5-Plate Kit
25-Plate Kit

K15155C-1
K15155C-2
K15155C-4



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MSD Toxicology Assays

Cardiac Injury Panel 2 (rat) Assay Kit

cTnl, cTnT, FABP3

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

ordering information

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Introduction

introduction

Troponin is a heterotrimer that regulates muscle contraction in skeletal and cardiac muscle (but not in smooth muscle). Troponin acts with intracellular calcium to control the interaction of actin and myosin filaments in striated muscle fibers. Though they perform similar functions, cardiac and skeletal troponins differ in sequence and can be differentiated in immunoassays.

The three subunits of troponin are:

- **Troponin T** is the subunit that interacts with tropomyosin to form the troponin-tropomyosin complex.
- **Troponin I** is an inhibitory subunit that prevents muscle contraction in the absence of calcium. It is responsible for the binding of the troponin-tropomyosin complex to actin. Troponin I exists in three isoforms: slow-twitch (striated) skeletal muscle, fast-twitch (striated) skeletal muscle, and cardiac muscle.
- **Troponin C** binds calcium, producing a conformational change in troponin I and activating the troponin-tropomyosin complex.

When muscle tissue is damaged, the troponin-tropomyosin complex breaks down and troponin I and troponin T are released into the blood. Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) can be readily distinguished from their skeletal muscle analogs allowing confirmation of cardiac muscle tissue damage over skeletal muscle tissue damage. Troponins are excellent biomarkers for myocardial injury in cardiotoxicity because of the demonstrated tissue-specificity of cardiac and skeletal troponins.

Fatty acid binding protein 3 (FABP3) is a monomeric protein that modulates the uptake of fatty acids in cells. Heart-type fatty acid binding protein is released into circulation after myocardial ischemia and necrosis. FABP3 is mostly present in heart and skeletal muscle but can also be found in brain, liver, and small intestine.

Principle of the Assay

principle of the assay

MSD[®] toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Cardiac Injury Panel 2 (rat) Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibodies for cTnI, cTnT, and FABP3 on spatially distinct spots. The user adds the sample and a solution containing the labeled detection antibodies—anti-cTnI, anti-cTnT, and anti-FABP3, labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibodies by bound analytes completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR[®] instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of cTnI, cTnT, and FABP3 present in the sample.

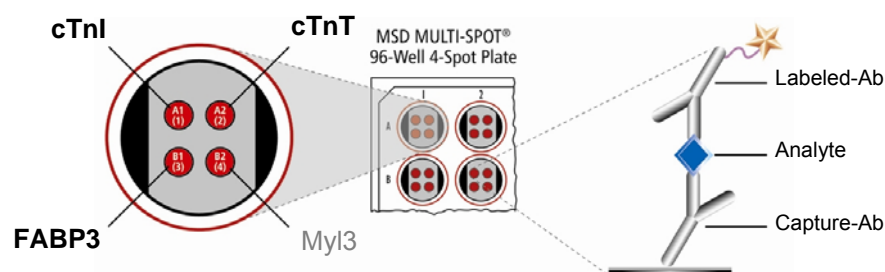


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K15155C-1	K15155C-2	K15155C-4
MULTI-SPOT® 96-well Cardiac Injury Panel 3 (rat) Plate* N45161A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-rat cTnI Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat cTnT Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat FABP3 Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Cardiac Injury Panel 3 (rat) Calibrator Blend (20X)* cTnI: 500 ng/mL cTnT: 2500 ng/mL FABP3: 2000 ng/mL Myl3: 1100 ng/mL	≤ -70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 7 R54BB-4 (5 mL) R54BB-3 (50 mL)	≤ -10°C	2 bottles (5 mL ea)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 30 R50AB-4 (25 mL)	≤ -10°C	1 bottle (25 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
25 mM DTT	≤ -10°C	1 vial (1 mL)	1 vial (1 mL)	5 vials (1 mL ea)
0.5 M EDTA pH 8.0	RT	1 bottle (4 mL)	1 bottle (4 mL)	5 bottles (4 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

***Note:** The Cardiac Injury Panel 3 (rat) Plate and Calibrator Blend (20X) are provided for both Cardiac Injury Panel 2 (rat) and Cardiac Injury Panel 3 (rat) kit.



Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

V Safety

s a f e t y

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

VI Reagent Preparation

r e a g e n t p r e p a r a t i o n

Bring all reagents to room temperature. *This is especially important for the Diluent 7, as some components are not soluble below room temperature.* Thaw the stock Calibrator blend on ice.

Important: Upon first thaw, separate Diluent 7 and Diluent 30 into aliquots appropriate to the size of your assay needs. These diluents can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Diluent 7 + Additives

For Cardiac Injury Panel 2, samples and Calibrators are diluted in Diluent 7 that contains EDTA and DTT. These two Additives must be added into the diluent by the user before each assay is carried out. EDTA and DTT additive stocks are provided at the concentrations in the table below.

For one plate combine:

- 540 μ L of EDTA stock solution
- 90 μ L of DTT stock solution
- 8370 μ L of Diluent 7

If sample dilution is not required, then a smaller volume of this reagent can be prepared.

Additive	Stock Conc.	Final Conc.
EDTA	500 mM (16.7X)	30 mM (1X)
DTT	25 mM (100X)	0.25 mM (1X)

Prepare Calibrator and Control Solutions

Calibrators for the Cardiac Injury Panel 2 (rat) are supplied at 20-fold higher concentration than the recommended highest Calibrator. For each assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The Calibrators are supplied as a blend. The stock Calibrator blend should be thawed and kept on ice, but should be added into diluent at room temperature to make the standard curve solutions (STD-01 through STD-08 below). The table below shows the concentrations of the 8-point standard curve:

Standard	cTnI (ng/mL)	cTnT (ng/mL)	FABP3 (ng/mL)	Dilution Factor
Stock Cal. Vial	500	2500	2000	
STD-01	25.0	125	100	20
STD-02	8.33	41.7	33.3	3
STD-03	2.78	13.9	11.1	3
STD-04	0.93	4.63	3.7	3
STD-05	0.31	1.54	1.23	3
STD-06	0.10	0.51	0.41	3
STD-07	0.03	0.17	0.14	3
STD-08	0	0	0	n/a

To prepare this 8-point standard curve for up to 5 replicates:

- 1) Prepare the highest Calibrator by adding 12 μ L of the Calibrator stock vial to 228 μ L of Diluent 7 + Additives.
- 2) Prepare the next Calibrator by transferring 80 μ L of the diluted Calibrator to 160 μ L of Diluent 7 + Additives. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 7 + Additives alone (i.e. zero Calibrator).

After preparation of the Calibrators at the concentrations above, incubate the Calibrator solutions without shaking for 30 minutes at room temperature prior to addition to the plate.

Dilution of Samples (optional)

Serum and plasma samples can be run neat or with 2-10X dilution, depending on the expected level of analytes in the samples. If sample dilution is required, to get the analyte levels into the detection range, Diluent 7 + Additives should be used to dilute the samples. For cardiac tissue lysates or homogenates, 50–500X dilution may be required. In this case, additional Diluent 7 is available for purchase. Diluted samples should be incubated without shaking at room temperature for 30 minutes prior to addition to the plate.

Prepare Detection Antibody Solution

The Detection Antibodies are provided as a 50X stock solution. The working Detection Antibody Solution should contain 1X as final concentration of each antibody.

In a 15 mL tube combine (per plate):

- 60 μ L of 50X SULFO-TAG Anti-rat cTnI Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat cTnT Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat FABP3 Antibody
- 2820 μ L of Diluent 30

Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibodies for the analytes shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

(Dilution of samples/Calibrators should be completed prior to Step 1)

- 1. Addition of Diluent 7 + Additives:** Dispense 25 μ L of Diluent 7 + Additives into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
- 2. Addition of the Sample or Calibrator:** Dispense 25 μ L of sample or Calibrator (which has been pre-incubated for 30 min following dilution with Diluent 7 + Additives) into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 3. Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25 μ L of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 4. Wash and Read:** Wash the plate 3X with PBS-T. Add 150 μ L of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD MULTI-SPOT plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

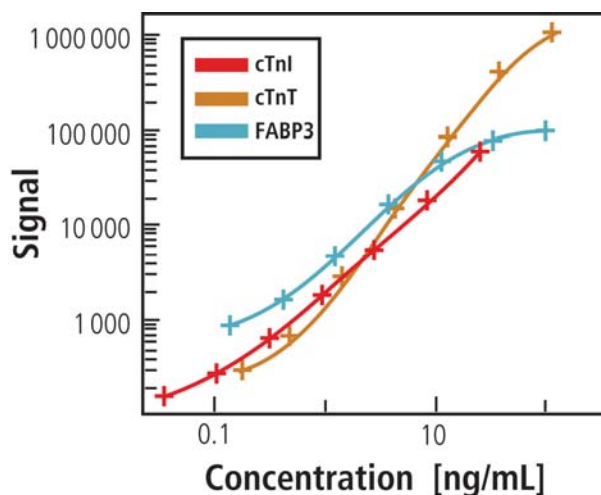
analysis of results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



cTnI		
Conc. (ng/mL)	Mean	%CV
0	87	7.5
0.03	160	7.0
0.10	277	3.3
0.31	652	3.3
0.93	1855	3.5
2.78	5540	6.1
8.33	18443	6.0
25.0	60948	8.2

cTnT		
Conc. (ng/mL)	Mean	%CV
0	145	18.8
0.17	295	11.2
0.51	694	18.0
1.54	2945	4.2
4.63	15218	5.3
13.9	87430	5.2
41.7	412064	5.3
125	1065029	3.9

FABP3		
Conc. (ng/mL)	Mean	%CV
0	567	7.1
0.14	882	4.5
0.41	1659	5.7
1.23	4720	4.5
3.70	16635	5.8
11.1	47437	3.6
33.3	78684	2.4
100	99544	4.0

X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero Calibrator.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%. For FABP3, the specification on percent recovery was widened to 75 – 125%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%. For FABP3, the specification on percent recovery was widened to 75 – 125%.

	cTnl	cTnT	FABP3
LLOD (ng/mL)	0.009	0.12	0.059
LLOQ (ng/mL)	0.098	0.49	0.39
ULOQ (ng/mL)	20	100	25

XI Precision

precision

Control samples were measured on 9 plates across three days. The controls were run in quadruplicate on each plate. These data were obtained on cardiac injury panel 3 kits.

	Control	Plates	Avg Conc. (ng/mL)	Intra-plate	Inter-plate
				Average %CV	%CV
cTnl	High	9	10.1	2.9	5.1
	Mid	9	1.52	2.6	4.0
	Low	9	0.29	2.1	4.3
cTnT	High	9	42.8	2.4	4.1
	Mid	9	7.75	1.4	3.2
	Low	9	1.20	2.2	4.5
FABP3	High	9	13.5	4.2	5.9
	Mid	9	7.58	2.3	4.0
	Low	9	2.49	1.6	2.5

XII Spike Recovery

spike recovery

Normal serum, heparin plasma, and EDTA plasma were spiked with the Calibrators at multiple values throughout the range of the assay. Spikes were made into neat samples. Values in italics for FABP3 were slightly above the ULOQ of 25 ng/mL. These data were obtained on MSD's Cardiac Injury Panel 3 (rat) Assay kit, which also includes Myl3 as an analyte².

cTnl	Spike Conc. (ng/mL)	Expected Conc. (ng/mL)	Measured Conc. (ng/mL)	%CV	% Recovery
Spiked Serum	2.5	3.27	3.26	5.17	100
	0.625	1.40	1.48	3.29	106
	0.156	0.93	0.97	1.06	105
	0		0.77	2.40	
Spiked Heparin Plasma	2.5	3.58	3.70	5.65	103
	0.625	1.71	1.78	8.02	104
	0.156	1.24	1.16	3.13	93
	0		1.08	4.30	
Spiked EDTA Plasma	2.5	3.49	3.33	1.96	96
	0.625	1.62	1.79	8.38	111
	0.156	1.15	1.11	0.69	97
	0		0.99	7.55	

cTnT	Spike Conc. (ng/mL)	Expected Conc. (ng/mL)	Measured Conc. (ng/mL)	%CV	% Recovery
Spiked Serum	12.5	13.17	11.56	1.66	88
	3.125	3.79	3.39	5.45	89
	0.781	1.45	1.29	1.27	89
	0		0.67	4.69	
Spiked Heparin Plasma	12.5	13.51	15.25	1.75	113
	3.125	4.13	4.64	3.84	112
	0.781	1.79	1.90	1.95	106
	0		1.01	8.22	
Spiked EDTA Plasma	12.5	13.08	10.89	1.39	83
	3.125	3.70	3.33	1.29	90
	0.781	1.36	1.35	3.30	99
	0		0.58	6.66	

FABP3	Spike Conc. (ng/mL)	Expected Conc. (ng/mL)	Measured Conc. (ng/mL)	%CV	% Recovery
Spiked Serum	10	24.92	26.97	13.64	108
	2.5	17.42	16.74	7.44	96
	0.625	15.54	16.25	3.61	105
	0		14.92	3.29	
Spiked Heparin Plasma	10	28.15	28.86	8.52	103
	2.5	20.65	20.08	4.68	97
	0.625	18.77	21.67	7.24	115
	0		18.15	4.09	
Spiked EDTA Plasma	10	27.68	27.70	7.07	100
	2.5	20.18	21.81	2.81	108
	0.625	18.31	19.01	3.07	104
	0		17.68	2.99	

² Catalog # K15161C-1 for 1-Plate Kit, K15161C-2 for 5-Plate Kit and K15161C-4 for 25-Plate Kit.

XIII Linearity

linearity

Multiple samples were tested for linearity of dilution. Recovery is calculated as the ratio of the adjusted concentration to the adjusted concentration of the previous dilution. Measurements that were outside of the quantitative range are shown in italics. For all measurements within the quantitative range, all the CV's on concentration were less than 15%.

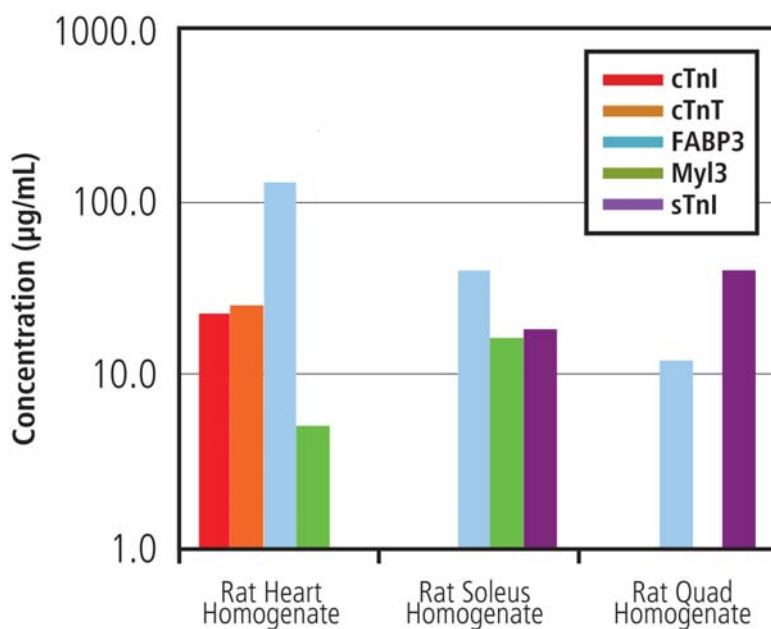
Sample	Fold Dilution	cTnl		cTnT		FABP3	
		Adjusted Conc. (ng.mL)	% Recovery	Adjusted Conc. (ng.mL)	% Recovery	Adjusted Conc. (ng.mL)	% Recovery
Pooled EDTA Plasma, Normal	Neat	3.71		2.85		79.76	
	2	3.35	90.1	2.97	104.3	66.89	> ULOQ
	4	3.03	90.6	2.62	88.3	55.33	NA
	8	2.83	93.4	2.38	< LLOQ	44.78	80.9
Pooled Heparin Plasma, Normal	Neat	3.55		2.60		49.53	
	2	3.44	96.8	2.40	92.4	52.13	> ULOQ
	4	3.10	90.1	2.24	93.2	49.93	NA
	8	2.92	94.1	1.99	< LLOQ	42.36	84.8
Serum from Normal Rat 1	Neat	1.29		0.86		22.33	
	2	1.25	96.4	0.86	< LLOQ	21.54	96.5
	4	1.07	86.1	0.72	< LLOQ	18.87	87.6
	8	0.89	82.9	0.22	< LLOQ	13.54	71.7
Serum from Normal Rat 2	Neat	0.72		0.60		19.16	
	2	0.69	96.0	0.54	< LLOQ	16.87	88.1
	4	0.65	94.5	0.43	< LLOQ	14.88	88.2
	8	0.62	< LLOQ	0.64	< LLOQ	13.08	87.9
Serum from Isoproterenol treated Rat 1	Neat	14.58		7.32		141.36	
	2	11.97	82.1	7.25	99.1	82.73	> ULOQ
	4	10.10	84.4	6.04	83.2	87.49	NA
	8	8.74	86.5	4.62	76.6	93.31	106.7
Serum from Isoproterenol treated Rat 2	Neat	20.43		11.28		68.88	
	2	15.97	78.2	10.64	94.3	75.31	> ULOQ
	4	13.28	83.2	9.28	87.2	95.74	NA
	8	10.72	80.7	7.05	76.0	90.49	94.5

XIV Specificity

specificity

Tissue homogenates from heart, fast twitch, and slow twitch muscle were tested at 100X, 1000X, and 10000X sample dilution on a custom 5-plex panel. The assays for cardiac troponins were positive for cardiac homogenates and negative for other muscle homogenates, demonstrating specificity for cardiac tissue. The assay for skeletal Troponin I was specific for fast and slow twitch skeletal muscle. The assay measured FABP3 in cardiac muscle and skeletal muscle. The slow twitch muscle was positive for Myl3, while approximately 200X less Myl3 was measured in fast twitch.

Sample Group	cTnl		cTnT		FABP3		Myl3		Skeletal Tnl	
	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)
Rat Heart Homogenate	1000	22.6	1000	25.1	10000	125.2	1000	5.0	100	< LLOD
Rat Soleus Homogenate (slow twitch)	100	< LLOD	100	< LLOD	10000	38.8	1000	16.4	1000	18.1
Rat Quad Homogenate (fast twitch)	100	< LLOD	100	< LLOD	1000	12.2	100	0.08	1000	40.9



XV Samples

s a m p l e s

Normal sample testing:

Serum, EDTA plasma, and heparin plasma samples collected from normal Sprague-Dawley rats were tested at 2-fold dilution on the Cardiac Injury Panel 3 (rat). Shown below are the median and range of concentrations for each sample set.

Sample	Statistic	cTnI	cTnT	FABP3
EDTA Plasma	Median (ng/ml)	1.85	1.21	35.01
	Range (ng/ml)	1.85 - 6.48	< 0.98 - 5.43	27.6 - > 50.0
	N	6	6	6
Heparin Plasma	Median (ng/ml)	1.89	1.38	26.57
	Range (ng/ml)	0.56 - 3.87	<0.98 - 3.37	10.9 - 42.5
	N	10	10	10
Serum	Median (ng/ml)	1.19	0.73	19.63
	Range (ng/ml)	0.26 - 2.49	< 0.98 - 2.19	5.14 - 27.0
	N	10	10	10

XVI Calibrators

c a l i b r a t o r s

Rat cardiac troponin I (cTnI), rat cardiac troponin T (cTnT), and rat fatty acid binding protein 3 (FABP3) were purified from rat heart tissue. Full-length recombinant rat myosin light chain 3 (MyI3) with an N-terminal 10xHis tag was expressed in *E. coli*. These analytes were calibrated against internal controls, diluted, and pooled to final concentrations of 500 ng/mL for cTnI, 2500 ng/mL for cTnT, 2000 ng/mL for FABP3, and 1100 ng/mL for MyI3 to make the Cardiac Injury Panel 3 (rat) Calibrator Blend. This blend is used for the Cardiac Injury Panel 2 (rat) Assay kit.

XVII References

r e f e r e n c e s

Babu L and Jaffe A S. Troponin: the biomarker of choice for the detection of cardiac injury. *CMAJ*.173(10): 1191-1202, 2005.

Summary Protocol

MSD 96-well MULTI-SPOT Cardiac Injury Panel 2 (rat) Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Cardiac Injury Panel 2 Assay.

Step 1 : Sample and Reagent Preparation

Bring appropriate diluents and plates to room temperature.

Diluent 7 + Additives should be prepared by diluting the provided DTT (100X) and EDTA (16.7X) stock solutions to 1X concentration in Diluent 7.

If necessary, samples should be diluted in Diluent 7 + Additives.

Prepare an 8-point standard curve using supplied Calibrators:

- The Calibrator Blend should be diluted in Diluent 7 + Additives.
- Dilute the stock Calibrator Blend 1:20 in Diluent 7 + Additives, then perform a series of 3-fold dilution steps and a no Calibrator blank.

Incubate the diluted Calibrators and diluted samples for 30 minutes without shaking at room temperature prior to addition to the plate.

Prepare Detection Antibody Solution by diluting the 50X Detection Antibodies into a 1X final concentration of each antibody. The Detection Antibodies should be diluted in 3.0 mL of Diluent 30 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting MSD Read Buffer T with deionized water.

Step 2 : Add Diluent 7 + Additives

Dispense 25 μ L/well Diluent 7 + Additives.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 30 min.

Step 3 : Add Sample or Calibrator

Dispense 25 μ L/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 μ L/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 μ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.

