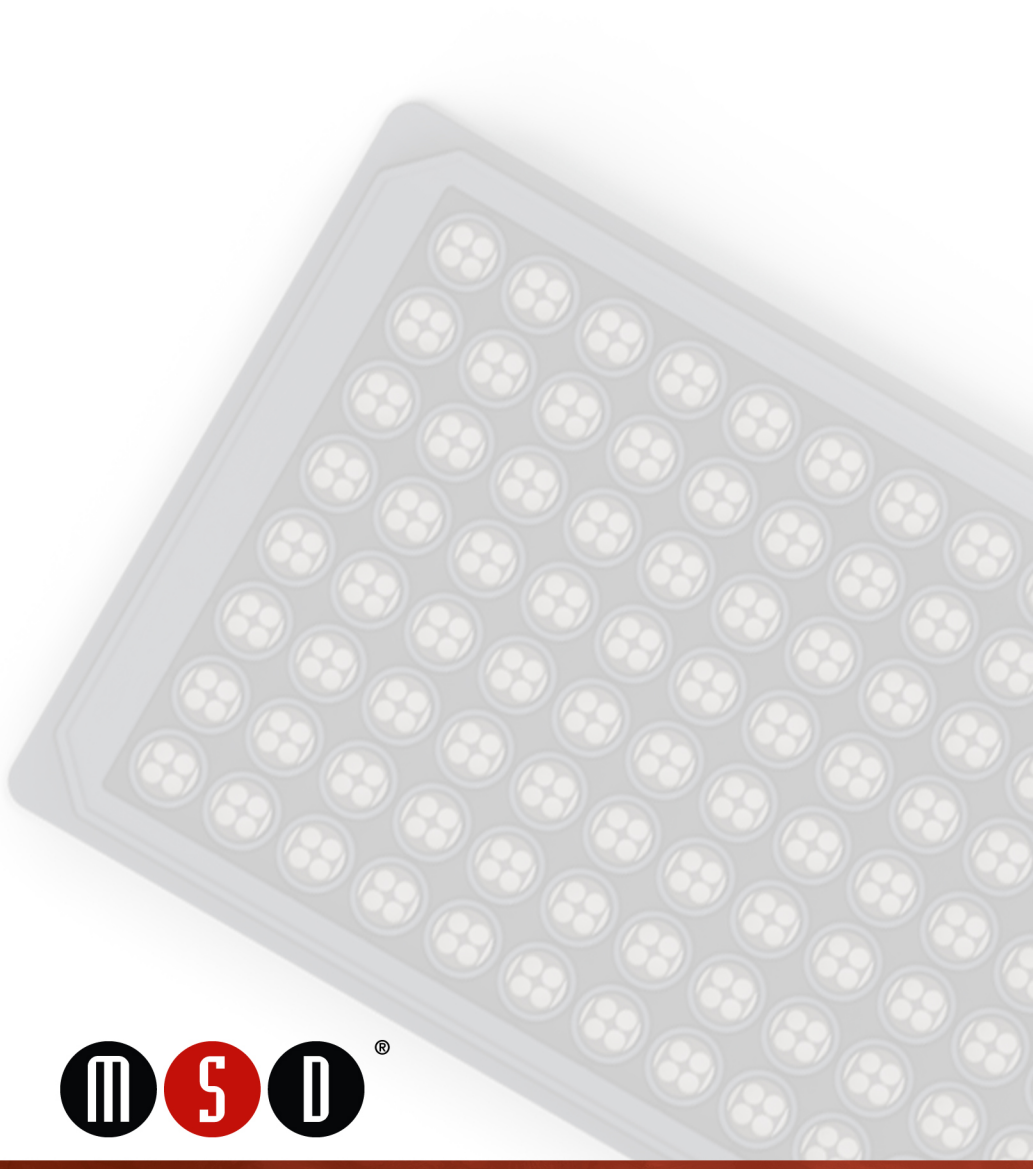
MSD® MULTI-SPOT Assay System

Cardiac Injury Panel 2 (rat) Kit

1-Plate Kit	K15155C-1
5-Plate Kit	K15155C-2
25-Plate Kit	K15155C-4



MSD Toxicology Assays

Cardiac Injury Panel 2 (rat) Kit

cTnI, cTnT, FABP3

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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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

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Cardiac Injury Panel 2 (rat) Kit are sandwich immunoassays (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent MSD SULFO-TAG™ labels 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 detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

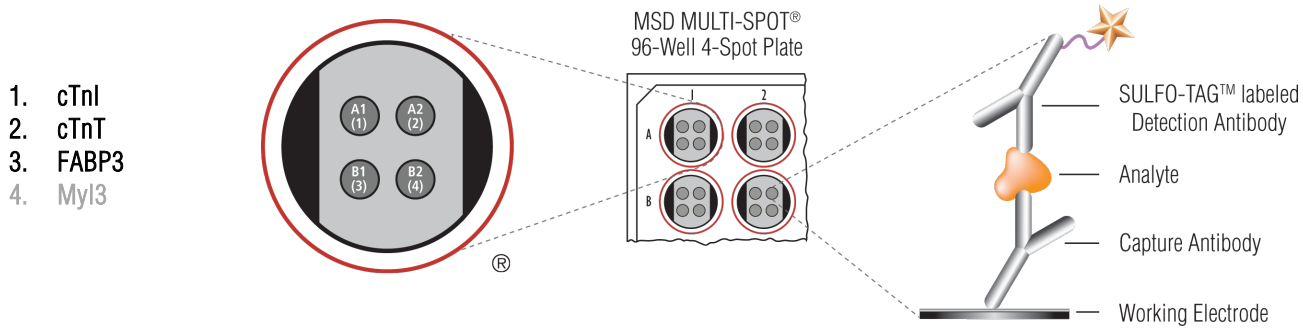


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Product Description	Storage	Catalog #	Size	Quantity per Kit		
				K15155C-1	K15155C-2	K15155C-4
MULTI-SPOT® 96-Well Cardiac Injury Panel 3 (rat) Plate	2–8°C	N45161A-1	4-spot	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat cTnI Antibody (50X)[1]	2–8°C	D23HR-2	75 µL	1 vial		
		D23HR-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-rat cTnt Antibody (50X)[1]	2–8°C	D23EF-2	75 µL	1 vial		
		D23EF-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-rat FABP3 Antibody (50X)[1]	2–8°C	D23HT-2	75 µL	1 vial		
		D23HT-3	375 µL		1 vial	5 vials
Cardiac Injury Panel 3 (rat) Calibrator Blend (20X)	≤-70°C	C0161-2	15 µL	1 vial	5 vials	25 vials
Diluent 7	≤-10°C	R54BB-4	5 mL	2 bottles		
		R54BB-3	50 mL		1 bottle	5 bottles
Diluent 30	≤-10°C	R50AB-4	25 mL	1 bottle	1 bottle	5 bottles
25 mM DTT	≤-10°C	NA	1 mL	1 vial	1 vial	5 vials
0.5 M EDTA pH 8.0	RT	NA	4 mL	1 bottle	1 bottle	5 bottles
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing or MSD Wash Buffer (Catalog No. R61AA-1)
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Deionized water
- ❑ Vortex mixer

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Best Practices

- Bring frozen diluent to room temperature in a 22-25°C water bath. Thaw frozen calibrator (when applicable) on wet ice.
- Prepare Calibrator Standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light (except for direct sunlight).
- Avoid bubbles in wells during all pipetting steps as they may lead to variable results. Bubbles introduced when adding Read Buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seal prior to reading the plate.
- Make sure that the read buffer is at room temperature when added to a plate.
- Do not shake the plate after adding the read buffer.
- To improve inter-plate precision, keep time intervals consistent between adding the read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. The uncoated wells of a partially used plate may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 7 and Diluent 30 into aliquots appropriate to the size of your needs before refreezing.

Prepare Diluent 7 + Additives

Samples and calibrators are diluted in Diluent 7 that contains added 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.

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

For one plate, combine:

- ❑ 540 µL of EDTA stock solution
- ❑ 90 µL of DTT stock solution
- ❑ 8,370 µL of Diluent 7

Prepare Calibrator Solutions

The Cardiac Injury Panel 2 (rat) Kit uses calibrator labeled as Cardiac Injury Panel 3 Calibrator. This Calibrator blend includes rat cTnI, cTnT, FABP3, and Myl3 proteins. The Calibrator blend is supplied at 20-fold higher concentration than the recommended highest Calibrator. For each assay, a 7-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock Calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions. For the actual concentration of each Calibrator in the blend, refer to the certificate of analysis (COA) supplied with the kit or available at www.mesoscale.com.

To prepare 7 standard solutions plus a zero calibrator blank for up to 5 replicates:

1) Prepare the highest standard by adding 10 µL of stock calibrator to 190 µL of Diluent 7 + Additives. Mix well.
2) Prepare the next standard by transferring 50 µL of the highest standard to 150 µL of Diluent 7 + Additives. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 7 + Additives as the blank.

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.

Dilute Samples

Serum and plasma samples should be run at 4-fold dilution. Diluent 7 + Additives should be used to dilute the samples. To perform sample dilution, add 25 µL of sample to 75 µL of Diluent 7 + Additives. For muscle tissue lysates or homogenates, a 500-fold dilution may be required. In this case, additional Diluent 7 can be purchased at www.mesoscale.com.

Diluted samples should be incubated at room temperature without shaking for 30 minutes prior to addition to the plate.

Prepare Detection Antibody Solution

MSD provides each detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat cTnI Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat cTnT Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat FABP3 Antibody
- ❑ 2,820 µL of Diluent 30

You may omit detection antibody for any analyte not being measured; add 60 µL of Diluent 30 for each omitted antibody.

Prepare Wash Buffer

MSD provides Wash Buffer as a 20X stock solution. The working solution is 1X. PBS + 0.05% Tween-20 can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

1X MSD Wash Buffer can be stored at room temperature for up to two weeks.

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For one plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Add Diluent 7 + Additives

- ❑ Add 25 µL of Diluent 7 + Additives solution to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 2: Add Sample or Calibrator

- ❑ Add 25 µL of sample or Calibrator (which has been pre-incubated for 30 min following dilution with Diluent 7 + Additives) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  You may prepare detection antibody solution during incubation.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  You may prepare diluted read buffer during incubation.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Analysis of Results

Run at least one set of calibrators in duplicate to generate the standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification without the need for dilution in many cases. The MSD DISCOVERY WORKBENCH® analysis software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Assay Qualification and Verification

The performance of this Kit meets levels of consistency and robustness as determined by methods based on the principles outlined in "Fit -for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al.[1]

Bioanalytical and functional characterizations of calibrators, antibodies and assay components are completed to allow for bridging of reagents between lots. This includes plate coating uniformity and reagent and component specificity testing for individual kit lots.

Control samples for specific matrices are designed and tested to meet the accuracy, precision and sensitivity criteria for a Kit that has completed the qualification process. Spike recovery and dilution linearity of endogenous samples, pooled and individual matrices are tested across the assay range.

- **Sensitivity, Range, and Curve Fitting**
  - Sample range and assay sensitivity are established from 4-PL fitted calibration curves with 1/Y2 weighting. Percent recovery of calibrators and controls between the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) must have a calculated concentration %CV of less than 20% and accuracy within 20% of the expected concentration.
  - The limits of quantification defined in the product insert are verified for each lot as part of the lot verification and quality control release.
- **Accuracy and Precision**

High, mid, and low controls made in matrix (need to be defined on a kit by kit basis) are run to measure accuracy and precision.

  - Qualification – Testing on multiple days (>6 days) and multiple runs per day for a total of 15–20 runs of complete kits. Precision is measured for the standard curve for intra- and inter-day %CV of less than 20%. %CV and accuracy of the controls are measured on all runs and must meet the kit specification as defined in the Certificate of Analysis (COA). The typical calculated concentration %CV specification is less than 20% and accuracy within 20% of expected concentration and a total error of less than 30%.
  - Verification – A multi-day (2–3 days), multiple runs per day for a total of 6–12 plates is performed as part of the release testing for each lot. The specifications for release are provided in the COA.
- **Robustness and Stability**

Freeze-thaw testing and accelerated stability studies performed during assay development (calibrators, antibodies, controls) are augmented with real-time stability studies on complete kits out to 18 months from the date of manufacture.

All acceptance criteria and verification conformance are defined in the COA for all kit lots. Presented below are representative data from the assay qualification for this assay that meets the criteria described above. The actual kit-specific standard curve and measured limits of quantification can be found in the COA enclosed with the kit.

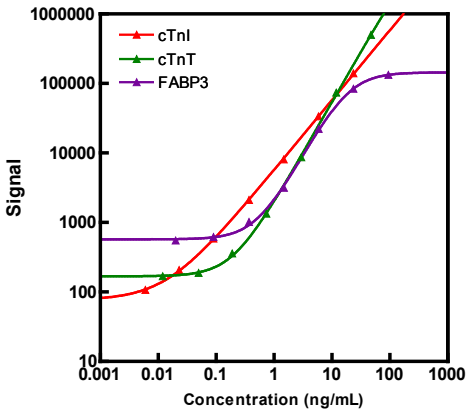
Typical Data

The following standard curve illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of two replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.

	cTnI	
Conc. (ng/mL)	Average Signal	%CV
0.0	85	19.5
0.006	107	7.2
0.023	207	3.5
0.09	585	4.6
0.37	2,110	3.3
1.47	8,056	3.3
5.88	33,509	4.4
23.5	139,742	2.1

	cTnT	
Conc. (ng/mL)	Average Signal	%CV
0.0	141	23.5
0.012	168	15.5
0.05	188	12.9
0.19	359	5.0
0.74	1,336	4.3
2.96	8,680	4.1
11.9	73,642	6.2
47.4	498,975	4.3

	FABP3	
Conc. (ng/mL)	Average Signal	%CV
0.0	510	5.3
0.02	549	3.8
0.09	618	3.4
0.37	1,019	4.4
1.48	3,145	7.1
5.9	22,184	3.2
23.6	83,674	2.7
94.5	132,515	2.3



Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator).

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantification (LLOQ) and upper limit of quantification (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%.

	cTnI	cTnT	FABP3
Average LLOD (ng/mL)	0.011	0.117	0.026
LLOQ (ng/mL)	0.098	0.49	0.39
ULOQ (ng/mL)	20	35	25

Precision

Control samples of high, mid, and low levels of each analyte were measured on each plate. For this study, Cardiac Injury Panel 3 (rat) kits were used.

The controls were run in quadruplicate on each of 9 plates run across 3 days.

Average Intra-plate %CV is the average %CV of the control replicates within an individual plate.

Inter-plate %CV is the variability of controls across 9 plates over 3 days.

	Control	Plates	Average Conc. (ng/mL)	Average Intra-plate %CV	Inter-plate %CV
cTnI	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
Myl3	High	9	25.0	3.6	5.1
	Mid	9	2.75	3.1	5.5
	Low	9	0.25	3.7	4.7

Dilution Linearity

To assess linearity, serum, EDTA plasma and heparin plasma samples were tested neat and at 2-fold, 4-fold, and 8-fold dilution using Cardiac Injury Panel 3 (rat). The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Measurements that were outside of the quantitative range are shown in italics. Percent recovery is calculated as the measured concentration divided by the concentration measured from the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

		cTnI			cTnT		
Sample	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum from Normal Rat 1	1	1.29	9.6		0.86	17.8	
	2	1.25	3.3	96.4	*0.86*	*4.8*	< LLOQ
	4	1.07	1.7	86.1	*0.72*	*8.3*	< LLOQ
	8	0.89	20.9	82.9	*0.22*	*173.2*	< LLOQ
Serum from Normal Rat 2	1	0.72	3.1		0.6	1.0	
	2	0.69	3.8	96.0	*0.54*	*9.0*	< LLOQ
	4	0.65	3.7	94.5	*0.43*	*100.4*	< LLOQ
	8	*0.62*	*5.9*	< LLOQ	*0.64*	*173.2*	< LLOQ
Serum from Isoproterenol treated Rat 1	1	14.58	3.1		7.32	2.1	
	2	11.97	1.1	82.1	7.25	2.8	99.1
	4	10.1	5.8	84.4	6.04	3.4	83.2
	8	8.74	4.5	86.5	4.62	3.0	76.6
Serum from Isoproterenol treated Rat 2	1	20.43	1.0		11.28	1.8	
	2	15.97	2.9	78.2	10.64	1.9	94.3
	4	13.28	4.5	83.2	9.28	2.0	87.2
	8	10.72	1.1	80.7	7.05	7.5	76.0
EDTA Plasma, Normal	1	3.71	8.1		2.85	3.2	
	2	3.35	10.9	90.1	2.97	7.1	104.3
	4	3.03	1.8	90.6	2.62	4.2	88.3
	8	2.83	4.8	93.4	*2.38*	*1.1*	< LLOQ
Heparin Plasma, Normal	1	3.55	5.1		2.6	2.9	
	2	3.44	5.9	96.8	2.4	2.6	92.4
	4	3.1	5.8	90.1	2.24	14.4	93.2
	8	2.92	3.0	94.1	*1.99*	*7.5*	< LLOQ

Dilution Linearity tables, continued.

		FABP3			Myl3		
Sample	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum from Normal Rat 1	1	22.33	13.6		0.41	16.8	
	2	21.54	11.1	96.5	0.41	4.0	100.2
	4	18.87	7.0	87.6	0.34	1.6	82
	8	13.54	18.2	71.7	*0.27*	*26.9*	< LLOQ
Serum from Normal Rat 2	1	19.16	4.1		0.54	4.6	
	2	16.87	6.3	88.1	0.45	5.4	84.1
	4	14.88	1.3	88.2	0.37	2.5	81.5
	8	13.08	4.4	87.9	*0.38*	*16.2*	< LLOQ
Serum from Isoproterenol treated Rat 1	1	*141.36*	*27.1*		6.73	7.0	
	2	*82.73*	*6.9*	> ULOQ	6.12	2.9	91.0
	4	87.49	4.0	NA	5.29	2.3	86.4
	8	93.31	1.6	106.7	4.33	1.7	81.9
Serum from Isoproterenol treated Rat 2	1	*68.88*	*30.4*		8.98	9.7	
	2	*75.31*	*4.1*	> ULOQ	7.46	3.8	83.1
	4	95.74	3.0	NA	6.84	1.6	91.7
	8	90.49	0.8	94.5	5.56	2.8	81.3
EDTA Plasma, Normal	1	*79.76*	*18.6*		1.53	7.9	
	2	*66.89*	*21.0*	> ULOQ	1.26	8.9	82.3
	4	55.33	0.9	NA	1.05	2.4	83.1
	8	44.78	1.7	80.9	0.95	4.0	90.8
Heparin Plasma, Normal	1	*49.53*	*12.4*		1.22	2.9	
	2	*52.13*	*10.9*	> ULOQ	1.21	3.7	98.8
	4	49.93	4.8	NA	1.1	5.2	90.8
	8	42.36	4.8	84.8	0.98	5.2	89.6

Spike Recovery

Normal serum, heparin plasma, and EDTA plasma were spiked with the Calibrators at multiple values throughout the range of the assay, and tested on Cardiac Injury Panel 3 (rat). Spikes were made into neat samples. Values in italics for FABP3 were slightly above the ULOQ of 25 ng/mL.

% Recovery = measured / expected x 100

	cTnI				cTnT			
Sample	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum	2.5	3.26	5.17	100	12.5	11.56	1.66	88
	0.625	1.48	3.29	106	3.125	3.39	5.45	89
	0.156	0.97	1.06	105	0.781	1.29	1.27	89
	0	0.77	2.40		0	0.67	4.69	
EDTA Plasma	2.5	3.33	1.96	96	12.5	10.89	1.39	83
	0.625	1.79	8.38	111	3.125	3.33	1.29	90
	0.156	1.11	0.69	97	0.781	1.35	3.30	99
	0	0.99	7.55		0	0.58	6.66	
Heparin Plasma	2.5	3.70	5.65	103	12.5	15.25	1.75	113
	0.625	1.78	8.02	104	3.125	4.64	3.84	112
	0.156	1.16	3.13	93	0.781	1.90	1.95	106
	0	1.08	4.30		0	1.01	8.22	

	FABP3				Myl3			
Sample	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum	10	*26.97*	*13.64*	*108*	5.5	6.45	4.79	110
	2.5	16.74	7.44	96	1.375	1.84	3.16	106
	0.625	16.25	3.61	105	0.344	0.77	2.26	108
	0	14.92	3.29		0	0.37	4.78	
EDTA Plasma	10	*27.70*	*7.07*	*100*	5.5	7.19	3.23	120
	2.5	21.81	2.81	108	1.375	2.11	4.49	114
	0.625	19.01	3.07	104	0.344	0.85	2.37	104
	0	17.68	2.99		0	0.47	6.25	
Heparin Plasma	10	*28.86*	*8.52*	*103*	5.5	6.60	1.68	112
	2.5	20.08	4.68	97	1.375	1.87	6.51	105
	0.625	21.67	7.24	115	0.344	0.72	4.65	96
	0	18.15	4.09		0	0.40	4.30	

Specificity

Specificity of the assays for individual Calibrators

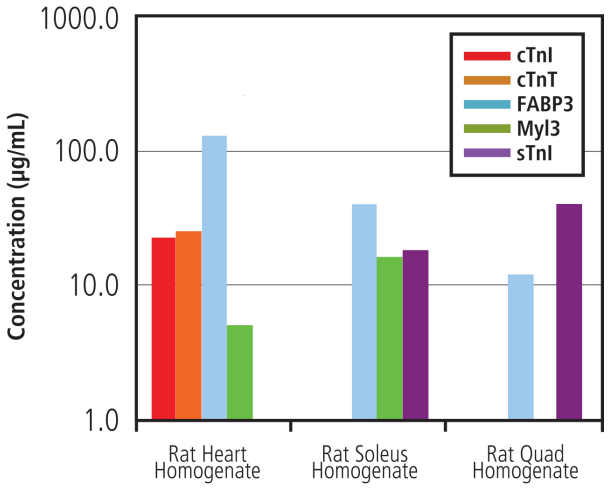
In order to assess specificity of the assays, the Cardiac Injury Panel 3 (rat) Kit was run with each calibrator at a high level, and blended detection antibodies. The table below shows the % cross-reactivity for each assay:

	Single Calibrator and Blended Detection Antibody % Cross-Reactivity			
Spot	**cTnI**	**cTnT**	**FABP3**	**Myl3**
cTnI	100	0.34	< 0.1	< 0.1
cTnT	3.0	100	0.19	< 0.1
FABP3	< 0.1	< 0.1	100	< 0.1
Myl3	< 0.1	< 0.1	< 0.1	100

Specificity of the assays for muscle homogenates

Tissue homogenates from heart, fast twitch, and slow twitch muscle were tested at 100-fold, 1000-fold, and 10,000-fold sample dilution on a 5-plex assay 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 200-fold less Myl3 was measured in fast twitch.

Sample Group	cTnI		cTnT		FABP3		Myl3		Skeletal TnI	
	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	1,000	22.6	1,000	25.1	10,000	125.2	1,000	5.0	100	< LLOD
Rat Soleus Homogenate (slow twitch)	100	< LLOD	100	< LLOD	10,000	38.8	1,000	16.4	1,000	18.1
Rat Quad Homogenate (fast twitch)	100	< LLOD	100	< LLOD	1,000	12.2	100	0.08	1,000	40.9



Tested Samples

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. Concentrations have been corrected for sample dilution.

Sample	Statistic	cTnI	cTnT	FABP3	Myl3
Serum	Median (ng/mL)	1.19	0.73	19.6	0.60
	Range (ng/mL)	0.26–2.49	<0.98–2.19	5.14–27.0	0.17–0.99
	N	10	10	10	10
EDTA Plasma	Median (ng/mL)	1.85	1.21	35.0	1.06
	Range (ng/mL)	1.85–6.48	<0.98–5.43	27.6–>50.0	0.63–2.68
	N	6	6	6	6
Heparin Plasma	Median (ng/mL)	1.89	1.38	26.6	0.83
	Range (ng/mL)	0.56–3.87	<0.98–3.37	10.9–42.5	0.34–1.94
	N	10	10	10	10

Assay Components

Calibrators

Rat cTnI, rat cTnT, and rat FABP3 were purified from rat heart tissue. Full-length recombinant rat Myl3 with an N-terminal 10xHis tag was expressed in *E. coli*. These analytes were pooled to make the Cardiac Injury Panel 3 (rat) Calibrator Blend. This Calibrator Blend is also used in the Cardiac Injury Panel 2 (rat) kit.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
cTnI	Mouse Monoclonal	Mouse Monoclonal
cTnT	Mouse Monoclonal	Mouse Monoclonal
FABP3	Chicken Polyclonal	Mouse Monoclonal
Myl3	Mouse Monoclonal	NA

References

1. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28.
2. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 2005 173(10):1191-1202, 2005.

Summary Protocol

Cardiac Injury Panel 2 (rat) Kit

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

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Diluent 7 + Additives by diluting the provided DTT (100X) and EDTA (16.7X) stock solutions to 1X concentration in Diluent 7.
- ❑ Prepare 8 calibration solutions using the supplied Calibrator:
  - o Dilute the stock Calibrator 20-fold in Diluent 7 + Additives.
  - o Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute serum and plasma samples 4-fold in Diluent 7 + Additives before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each stock detection antibody 50-fold in Diluent 30.
- ❑ Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T 4-fold with deionized water.

STEP 1: Add Diluent 7 + Additives

- ❑ Add 25 µL/well of Diluent 7 + Additives.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 30 minutes.

STEP 2: Add Sample or Calibrator

- ❑ Add 25 µL/well of sample or Calibrator.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 4: Wash and Read Plate

- ❑ Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.

Plate Diagrams