

Meso Scale Discovery[®]

MULTI-SPOT[®] Assay System

Muscle Injury Panel 1 (rat) Assay Kit

1-Plate Kit

K15181C-1

5-Plate Kit

K15181C-2

25-Plate Kit

K15181C-4

This product insert should be used with kit lot # K0040050



Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Di



MSD Toxicology Assays

Muscle Injury Panel 1 (rat) Assay Kit

cTnl, cTnT, sTnl, FABP3, Myl3

This product insert must be read in its entirety before use.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

Meso Scale Discovery, Meso Scale Diagnostics, www.mesoscale.com, MSD, MSD (design), Discovery Workbench, Quickplex, Multi-Array, Multi-Spot, Sulfo-Tag and Sector are trademarks of Meso Scale Diagnostics, LLC.

© 2009 Meso Scale Discovery a division of Meso Scale Diagnostics, LLC. All rights reserved.

Table of Contents

table of contents

Introduction.....	4
Principle of the Assay	5
Reagents Supplied	6
Required Material and Equipment – not supplied.....	7
Safety	7
Reagent Preparation	7
Assay Protocol.....	10
Analysis of Results	10
Typical Standard Curve	11
Sensitivity	12
Precision.....	12
Spike Recovery	13
Linearity	14
Specificity	15
Samples.....	17
Calibrators	17
References	17
Summary Protocol	19
Plate Diagrams	21

Ordering Information

ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

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.

Myosin light chain 3 (Myl3) is an essential light chain of the myosin molecule found in cardiac and slow-twitch skeletal muscle. Myosin is a hexamer ATPase motor protein that is a major constituent of thick muscle filament. The myosin molecule consists of a head domain that “walks” along the actin chain to contract the muscle and a tail domain that is responsible for binding the myosin to its cargo. Two heavy chain subunits intertwine to form the head and tail domains and four light chain subunits—two regulatory light chains with phosphorylation sites (encoded by the MYL2 genes), and two essential light chains (encoded by the MYL3 genes). These light chains bind the heavy chains together in the neck region between the head and tail domains. After damage to muscle tissue, myosin breaks down and Myl3 becomes elevated in the blood. Myl3 can be used in conjunction with other toxicity biomarkers to confirm cardiac and slow twitch skeletal muscle injury.

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 Muscle Injury Panel 1 (rat) Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibodies for cTnI, cTnT, sTnI, FABP3, and Myl3 on spatially distinct spots. The user adds the sample and a solution containing the labeled detection antibodies—anti-cTnI, anti-cTnT, anti-sTnI, anti-FABP3, and anti-Myl3 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, sTnI, FABP3 and Myl3 present in the sample.

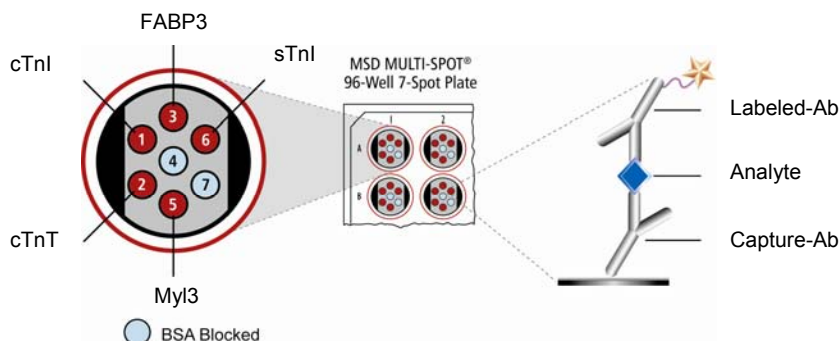


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K15181C-1	K15181C-2	K15181C-4
MULTI-SPOT [®] 96-well Muscle Injury Panel 1 (rat) Plate N75181A-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)
SULFO-TAG Anti-rat Myl3 Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat sTnI Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Muscle Injury Panel 1 (rat) Calibrator Blend (20X) Lot A0040021 cTnI: 656 ng/mL cTnT: 2760 ng/mL sTnI: 4380 ng/mL FABP3: 1858 ng/mL Myl3: 1068 ng/mL	≤ -70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 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)

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

IV 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 μ L into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

V Safety

safety

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. Material Safety Data Sheets are available upon request.

VI Reagent Preparation

reagent preparation

Bring all plates and diluents 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 Muscle Injury Panel 1, 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 Solutions

Calibrators for the Muscle Injury Panel 1 (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 concentrations provided are for Calibrator Lot A0040021. 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	cTnl (ng/mL)	cTnT (ng/mL)	FABP3 (ng/mL)	MyI3 (ng/mL)	sTnl (ng/mL)	Dilution Factor
Stock Cal. Vial	656	2760	1858	1068	4380	
STD-01	32.8	138	92.9	53.4	219	20
STD-02	10.9	46.0	31.0	17.8	73.0	3
STD-03	3.64	15.3	10.3	5.93	24.3	3
STD-04	1.21	5.11	3.44	1.98	8.11	3
STD-05	0.40	1.70	1.15	0.66	2.70	3
STD-06	0.13	0.57	0.38	0.22	0.90	3
STD-07	0.04	0.19	0.13	0.07	0.30	3
STD-08	0	0	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

For serum and plasma from normal rats, no dilution is necessary. However, for rats that have suffered a muscle injury, 2–10X dilution of serum or plasma samples may be necessary. If sample dilution is required to get the analyte levels into the detection range, Diluent 7 + Additives should be used to dilute the samples.

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
- 60 μ L of 50X SULFO-TAG Anti-rat Myl3 Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat sTnI Antibody
- 2700 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 antibody 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 μL 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 μL 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 μL 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 μL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates should 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 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.

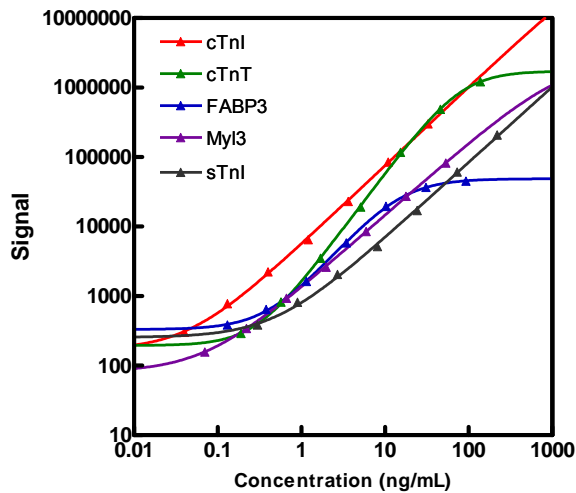
IX Typical Standard Curve

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.

cTnl			cTnT		
Conc. (ng/mL)	Average Signal	%CV	Conc. (ng/mL)	Average Signal	%CV
0	63	27.9	0	106	14.8
0.04	301	6.2	0.19	289	4.9
0.13	780	3.5	0.57	820	5.0
0.40	2229	4.6	1.70	3503	4.9
1.21	6476	3.9	5.11	19061	4.6
3.64	22919	3.5	15.3	117334	5.0
10.9	84983	3.8	46.0	489131	4.2
32.8	300781	5.4	138	1215794	4.7

FABP3			MyI3		
Conc. (ng/mL)	Average Signal	%CV	Conc. (ng/mL)	Average Signal	%CV
0	268	7.1	0	45	27.5
0.13	384	4.9	0.07	157	8.5
0.38	641	6.4	0.22	344	5.8
1.15	1630	5.3	0.66	933	5.6
3.44	5827	4.9	1.98	2596	5.2
10.3	19617	4.2	5.93	8471	10.2
31.0	36688	6.2	17.8	27314	4.1
92.9	45176	6.9	53.4	82420	5.5



sTnl		
Conc. (ng/mL)	Average Signal	%CV
0	184	19.3
0.30	384	5.8
0.90	816	3.1
2.70	2055	2.8
8.11	5162	2.8
24.3	17092	3.5
73.0	60786	3.1
219	207728	4.6

X Sensitivity

sensitivity

The lower limit of detection (LLOD) is measured as the concentration at 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 the FABP3 and Myl3 assay's LLOQ, the %CV of the calculated concentration is less than 25% and the percent recovery of the standard is between 75% and 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 the FABP3 assay's ULOQ, the %CV of the calculated concentration is less than 25% and the percent recovery of the standard is between 75% and 125%.

	cTnl (ng/mL)	cTnT (ng/mL)	FABP3 (ng/mL)	Myl3 (ng/mL)	sTnl (ng/mL)
LLOD	0.010	0.079	0.069	0.023	0.148
LLOQ	0.098	0.488	0.391	0.054	0.781
ULOQ	20.0	100	15.0	44.0	160

XI Precision

precision

Control samples were measured on 9 plates across 3 days. The controls were run in triplicate or quadruplicate on each plate. Normal rat serum, rat soleus homogenate, and assay Calibrators are used to make control samples. The high control contains 25% normal rat serum and Calibrators. The mid control contains rat soleus homogenate and Calibrators. The low control contains only the assay Calibrators.

The average intra-plate %CV and inter-plate %CV of the concentrations are shown below.

	Control	Plates	Average Conc. (ng/mL)	Intra-plate	Inter-plate
				Average %CV	%CV
cTnl	High	9	9.36	3.8	5.9
	Mid	9	1.31	3.9	6.0
	Low	9	0.28	4.0	5.1
cTnT	High	9	35.4	3.9	5.0
	Mid	9	6.46	2.5	4.0
	Low	9	1.06	3.3	4.6
FABP3	High	9	13.9	5.9	7.5
	Mid	9	8.44	5.5	7.6
	Low	9	2.85	2.8	4.8
Myl3	High	9	30.3	5.9	7.0
	Mid	9	3.01	3.8	6.9
	Low	9	0.32	6.0	7.1
sTnl	High	9	108	4.3	5.8
	Mid	9	17.4	2.4	3.1
	Low	9	2.88	3.5	10.5

XII Spike Recovery

spike recovery

Pooled normal rat 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 are outside of the range of quantitation.

% Recovery = measured / expected x 100

cTnI	Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	2.50	5.13	10.1	116
	0.83	3.22	8.6	116
	0.28	2.47	3.8	111
	0	1.94	5.3	
Heparin Plasma	2.50	6.42	2.7	134
	0.83	3.83	7.3	123
	0.28	2.69	4.3	105
	0	2.28	4.0	
EDTA Plasma	2.50	3.37	1.1	128
	0.83	1.12	2.1	116
	0.28	0.44	4.0	108
	0	0.13	6.2	

cTnT	Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	12.5	10.1	4.1	77
	4.17	4.09	4.4	86
	1.39	1.72	3.6	86
	0	0.60	2.8	
Heparin Plasma	12.5	13.4	0.7	100
	4.17	5.38	5.5	104
	1.39	2.26	1.2	95
	0	0.99	4.2	
EDTA Plasma	12.5	11.2	2.9	89
	4.17	3.27	2.3	78
	1.39	0.99	3.5	71
	0	0.02	115	

FABP3	Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	10.0	16.0	12.9	114
	3.33	8.28	15.3	113
	1.11	5.03	16.2	98
	0	4.01	3.9	
Heparin Plasma	10.0	32.6	14.6	90
	3.33	29.2	26.2	99
	1.11	23.8	22.8	87
	0	26.2	15.3	
EDTA Plasma	10.0	13.6	15.1	100
	3.33	6.54	15.2	93
	1.11	4.35	12.0	91
	0	3.66	12.9	

Myl3	Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	5.50	10.4	14.4	115
	1.83	6.47	4.8	120
	0.61	4.36	5.8	105
	0	3.55	4.9	
Heparin Plasma	5.50	12.8	3.1	138
	1.83	7.12	2.6	128
	0.61	4.66	2.8	107
	0	3.73	3.4	
EDTA Plasma	5.50	12.9	0.9	136
	1.83	7.65	8.1	131
	0.61	4.91	7.1	107
	0	4.00	2.0	

sTnI	Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	20.0	52.5	7.1	101
	6.67	41.8	2.0	109
	2.22	34.1	3.4	100
	0	31.7	4.6	
Heparin Plasma	20.0	57.5	1.3	110
	6.67	41.8	1.2	107
	2.22	34.5	2.9	100
	0	32.3	3.0	
EDTA Plasma	20.0	54.1	2.7	107
	6.67	39.9	1.0	108
	2.22	32.3	3.8	99
	0	30.4	1.7	

XIII Linearity

linearity

To assess linearity, rat serum, EDTA plasma, and heparin plasma samples from Sprague-Dawley rats were tested undiluted (1) and at 2-fold, 4-fold, and 8-fold dilution. The “serum 2” sample was taken from a rat treated with isoproterenol. All other samples were taken from normal rats. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

cTnI	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 1	1	0.986	3.8	
	2	0.992	1.4	101
	4	0.896	3.6	90
	8	0.827	5.1	92
Serum 2	1	31.16	1.9	
	2	28.49	2.9	91
	4	24.48	2.9	86
	8	20.16	9.4	82
Heparin Plasma 1	1	2.12	2.2	
	2	2.07	2.2	98
	4	1.97	8.0	96
	8	1.76	8.3	89
EDTA Plasma 1	1	0.370	13.0	
	2	0.401	8.4	108
	4	0.439	2.3	109
	8	< LLOQ	-	-

cTnT	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 1	1	0.520	0.1	
	2	< LLOQ	-	-
	4	< LLOQ	-	-
	8	< LLOQ	-	-
Serum 2	1	2.63	9.2	
	2	3.06	1.2	116
	4	3.29	0.8	107
	8	< LLOQ	-	-
Heparin Plasma 1	1	0.984	2.9	
	2	1.00	0.3	101
	4	< LLOQ	-	-
	8	< LLOQ	-	-
EDTA Plasma 1	1	< LLOQ	-	
	2	< LLOQ	-	-
	4	< LLOQ	-	-
	8	< LLOQ	-	-

FABP3	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 3	1	> ULOQ		
	2	22.4	11.6	-
	4	17.3	11.6	77
	8	15.1	15.3	88
Serum 4	1	> ULOQ		
	2	16.7	20.4	-
	4	14.3	11.0	86
	8	11.8	16.0	83
Heparin Plasma 2	1	> ULOQ		
	2	21.3	26.5	-
	4	19.7	11.9	93
	8	20.5	4.4	104
EDTA Plasma 2	1	3.99	14.0	
	2	3.72	28.5	93
	4	3.74	13.9	100
	8	< LLOQ	-	-

MyI3	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 1	1	5.18	0.8	
	2	4.44	2.2	86
	4	4.09	6.0	92
	8	3.92	5.9	96
Serum 2	1	15.1	5.0	
	2	14.2	5.3	94
	4	13.3	9.9	94
	8	11.8	10.6	89
Heparin Plasma 1	1	3.54	11.4	
	2	3.61	1.3	102
	4	3.47	2.9	96
	8	3.71	11.5	107
EDTA Plasma 1	1	2.95	19.7	
	2	2.90	10.2	99
	4	2.74	4.5	94
	8	2.44	0.6	89

sTnI	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 1	1	30.8	2.2	
	2	24.5	0.6	79
	4	21.3	5.2	87
	8	19.7	3.1	92
Serum 2	1	3.93	7.6	
	2	3.32	10.6	85
	4	< LLOQ	-	-
	8	< LLOQ	-	-
Heparin Plasma 1	1	32.7	0.2	
	2	28.6	2.0	88
	4	27.1	7.3	95
	8	26.2	3.2	97
EDTA Plasma 1	1	22.8	14.9	
	2	23.8	5.4	104
	4	21.7	1.6	91
	8	20.0	6.8	92

XIV Specificity

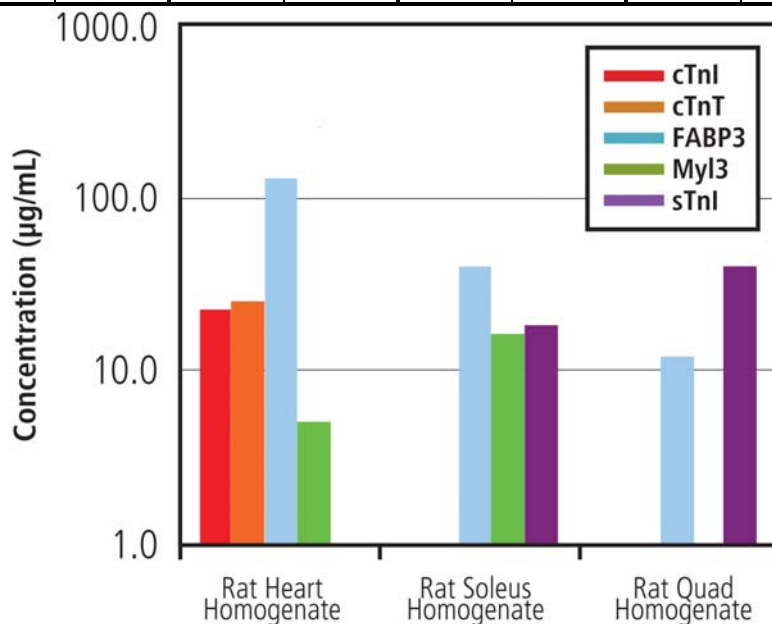
specificity

The assays on the Muscle Injury Panel 1 (rat) are all highly specific for their respective analytes. Specificity was demonstrated by testing rat muscle homogenates, and by running the assay with single Calibrators and single detection antibodies. Results are detailed in the sections below.

Testing of Rat Muscle Homogenates:

Tissue homogenates from heart, fast twitch, and slow twitch muscle were tested at 100X, 1000X, and 10000X sample dilution. 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		sTnl	
	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



Specificity of the assays for individual Calibrators:

The Muscle Injury Panel 1 assay was run with each single Calibrator at a high level in order to assess specificity. The table below shows the % cross-reactivity of each assay for each Calibrator. The % cross-reactivity is calculated as the ratio of the non-specific signal to the specific signal.

Blended Detection Antibodies and Single Calibrator % Cross-Reactivity					
Spot	cTnl	cTnT	FABP3	MyI3	sTnl
cTnl	100	0.55	< 0.1	< 0.1	< 0.1
cTnT	0.19	100	< 0.1	< 0.1	1.93
FABP3	< 0.1	< 0.1	100	< 0.1	< 0.1
MyI3	< 0.1	< 0.1	< 0.1	100	< 0.1
sTnl	< 0.1	< 0.1	< 0.1	< 0.1	100

Specificity of the Detection Antibodies:

In order to assess specificity of the detection antibodies, the Muscle Injury Panel 1 panel was run with blended Calibrator diluted to STD-02, and single detection antibodies. The table below shows the % cross-reactivity for each individual detection antibody. The data shows that the presence of cTnl Calibrator on the cTnl spot is reported out by the cTnl, cTnT, and sTnl detection antibodies.

Blended Calibrator and Single Detection Antibody % Cross-Reactivity					
Spot	cTnl	cTnT	FABP3	MyI3	sTnl
cTnl	100	< 0.1	< 0.1	< 0.1	50.9
cTnT	0.44	100	< 0.1	0.17	7.89
FABP3	< 0.1	< 0.1	100	< 0.1	0.11
MyI3	< 0.1	< 0.1	< 0.1	100	0.11
sTnl	0.24	< 0.1	< 0.1	< 0.1	100

While these detection antibodies are not highly specific, the assays themselves are highly specific due to the specificity of the capture antibodies.

XV Samples

s a m p l e s

Serum, heparin plasma, and EDTA plasma samples collected from normal Sprague-Dawley rats were tested at 2-fold dilution on the Muscle Injury Panel 1 (rat). Shown below are the median and range of concentrations for each sample set. Skeletal Troponin I was below the quantitative range for all samples.

		cTnI	cTnT	FABP3	Myl3	sTnI
Serum	Median (ng/mL)	1.59	0.83	19.84	0.61	< 1.56
	Range (ng/mL)	0.44 - 3.53	< 0.976 - 2.04	5.28 - > 30	0.25 - 1.05	< 1.56
	# of samples	10	10	10	10	10
Heparin Plasma	Median (ng/mL)	1.81	1.06	28.35	0.55	< 1.56
	Range (ng/mL)	0.37 - 4.10	< 0.976 - 2.79	4.12 - > 30	0.16 - 1.16	< 1.56
	# of samples	10	10	10	10	10
EDTA Plasma	Median (ng/mL)	2.31	1.28	35.63	0.81	< 1.56
	Range (ng/mL)	1.45 - 4.05	< 0.976 - 2.80	19.6 - > 30	0.43 - 1.17	< 1.56
	# of samples	10	10	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. Rat skeletal troponin I was purified from rat skeletal muscle. Full-length recombinant rat myosin light chain 3 (Myl3) with an N-terminal 10xHis tag was expressed in *E. coli*. These analytes were calibrated against internal controls, diluted, and pooled to make the Muscle Injury Panel 1 (rat) Calibrator Blend.

XVII References

r e f e r e n c e s

Babuin 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 Muscle Injury Panel 1 (rat) Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Muscle Injury Panel 1 Assay.

Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator on ice.

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 to a 1X final concentration of each antibody in 3.0 mL of Diluent 30 (per plate).

Prepare 20 mL of 1X Read Buffer T by diluting 4X 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.

Immediately analyze plate on SECTOR instrument.

