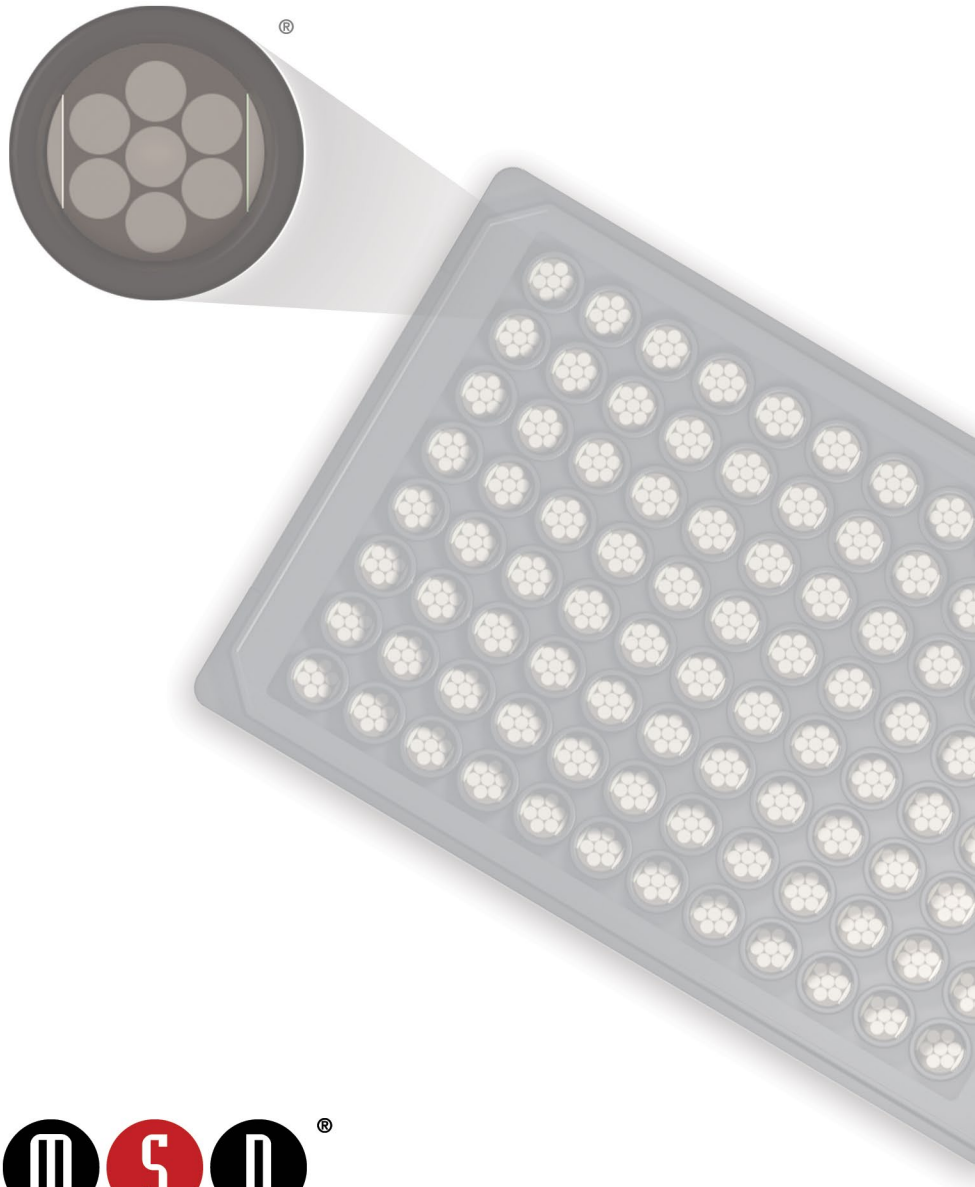
MSD® MULTI-SPOT Assay System

Muscle Injury Panel 3 (mouse) Kit

cTnI, FABP3, Myl3, Troponin I (fast-twitch)

T-PLEX®



	Catalog No
Multiplex Kit	K15186C

MSD®

MSD Toxicology Assays

Muscle Injury Panel 3 (mouse) Kit

cTnI, FABP3, Myl3, Troponin I (fast-twitch)

For use with mouse serum and plasma samples.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®

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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. 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.[1] Troponin I exists in 3 isoforms which are found in slow-twitch (striated) skeletal muscle, fast-twitch (striated) skeletal muscle, and cardiac muscle (**cTnI**).[2] Troponins are excellent biomarkers for myocardial injury in cardiotoxicity because of their demonstrated tissue specificity.[3]

Myosin light chain 3 (Myl3) is an essential light chain of the myosin molecule that is found in cardiac and slow-twitch skeletal muscle.[4] Myosin is a hexamer ATPase motor protein and a major constituent of thick muscle filament. It 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 4 light chain subunits, 2 regulatory light chains with phosphorylation sites (encoded by the MYL2 genes) and 2 essential light chains (encoded by the MYL3 genes)[5], 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.[6]

Fatty acid binding protein 3 (FABP3) is a monomeric protein that modulates the uptake of fatty acids in cells.[7] Heart-type fatty acid binding protein is released into circulation after myocardial ischemia and necrosis.[8] 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 Muscle Injury Panel 3 (mouse) Kit are sandwich immunoassays (Figure 1). MSD provides a plate precoated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) throughout 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. This panel has been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[9]

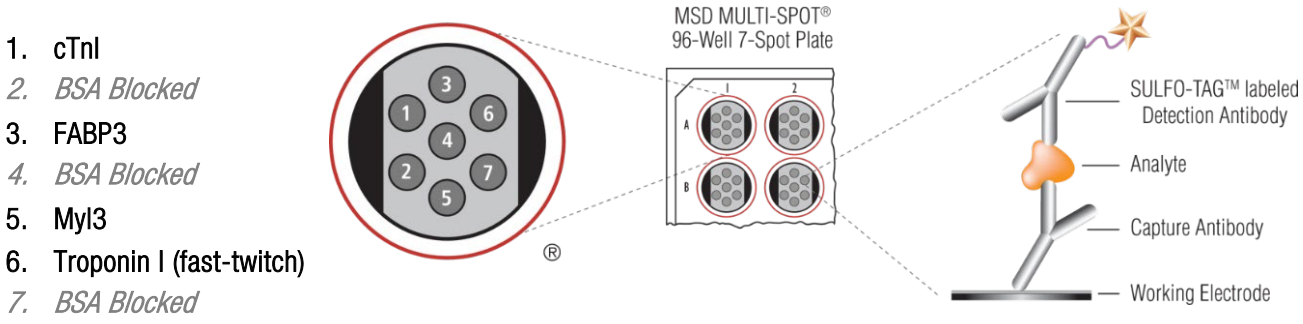

Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Kit Components

Reagents Supplied

Kit reagents are listed in Table 1.

Table 1. Reagents that are supplied with the Kit

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Muscle Injury Panel 3 (mouse) Plate	2–8 °C	N75186A-1	7-spot	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant.
Muscle Injury Panel 3 (mouse) Calibrator Blend (20X)	≤–70 °C	—	1 vial (20 µL)	1 vial	5 vials	25 vials	Four recombinant mouse proteins in diluent, buffered, and frozen. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
Anti-ms cTnI Antibody (50X)	2–8 °C	—	75 µL	1 vial	—	—	SULFO-TAG™ conjugated antibody
			375 µL	—	1 vial	5 vials	
Anti-ms FABP3 Antibody (50X)	2–8 °C	—	75 µL	1 vial	—	—	SULFO-TAG conjugated antibody
			375 µL	—	1 vial	5 vials	
Anti-ms Myl3 Antibody (50X)	2–8 °C	—	75 µL	1 vial	—	—	SULFO-TAG conjugated antibody
			375 µL	—	1 vial	5 vials	
Anti-ms Troponin I (fast-twitch) Antibody (50X)	2–8 °C	—	75 µL	1 vial	—	—	SULFO-TAG conjugated antibody
			375 µL	—	1 vial	5 vials	
Diluent 30	≤–10 °C	R50AB-4	25 mL	1 bottle	1 bottle	5 bottles	Diluent for detection antibody
Diluent 33	≤–10 °C	R50AD-4	5 mL	2 bottles	—	—	Diluent for samples and Calibrators
		R50AD-3	50 mL	—	1 bottle	5 bottles	
DTT (25 mM)	≤–10 °C	—	1 mL	1 vial	1 vial	5 vials	Diluent 33 additives
EDTA pH 8.0 (0.5M)	RT	—	4 mL	1 bottle	1 bottle	5 bottles	Diluent 33 additives
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	Buffer to catalyze the electro-chemiluminescent reaction

RT = room temperature
dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid-handling equipment suitable for dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) or phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Adhesive plate seals
- ❑ 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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
- Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs.
- Bring frozen diluents to room temperature in a 20–26 °C water bath before use. If a controlled water bath is not available, thaw at room temperature. Diluents may also be thawed overnight at 2–8 °C. Thaw other reagents on wet ice and use as directed without delay.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid prolonged exposure of 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.
- Plate shaking should be vigorous, with a rotary motion between 300–1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping the sample or detection antibody solution in the plate.
- Remove the plate seals before reading the plate.
- Read buffer should be at room temperature (20–26 ºC) before adding it to the plate.
- Do not shake the plate after adding read buffer.
- Keep time intervals consistent between the addition of read buffer and reading the plate to improve interplate precision. It is recommended that an MSD instrument be prepared to read a plate before adding read buffer. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Reagent Preparation

Bring all the reagents to room temperature and refer to the Best Practices section (page 8) before beginning the protocol.

Important: Upon the first thaw, aliquot Diluent 30 and Diluent 33 into suitably sized aliquots before refreezing.

Prepare Diluent 33 plus Additives

For the Muscle Injury Panel 3, samples and calibrators must be diluted in Diluent 33 to which EDTA and DTT have been added. EDTA and DTT additive stocks are provided at the concentrations shown in the table below.

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

For one plate, combine the following additives and Diluent 33.

- ❑ 90 µL of DTT stock solution
- ❑ 540 µL of EDTA stock solution
- ❑ 8,370 µL of Diluent 33

Prepare Calibration Dilutions

MSD supplies a blended calibrator for the Muscle Injury Panel 3 (mouse) Kit at a 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve 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 it on ice, then add to diluent at room temperature to make the standard curve solutions. To view the actual concentration of each calibrator in the blend, refer to the certificate of analysis (COA) supplied with the kit. You may also find a copy of the lot-specific COA at www.mesoscale.com by entering K15186C in the search box.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

1) Prepare the highest calibrator (Calibrator 1) by adding 15 µL of stock calibrator to 285 µL of Diluent 33 plus additives. Mix well by vortexing.
2) Prepare the next calibrator by transferring 60 µL of the highest standard to 180 µL of Diluent 33 plus additives. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 33 plus additives as the zero calibrator.

Dilute Samples

For mouse serum and plasma samples, MSD recommends a 4-fold dilution in Diluent 33 plus additives; however, you may adjust dilution factors for the sample set under investigation. For example, when running 4-fold diluted samples in duplicate, add 25 µL of sample to 75 µL of Diluent 33 plus additives. We recommend running at least two replicates per sample. Sample collection methods may affect the FABP3 endogenous levels. The kit includes diluent sufficient for running samples in duplicate. Additional diluent can be purchased at www.mesoscale.com.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use.

For one plate, combine the following detection antibodies and add to 2,760 µL of Diluent 30.

- ❑ 60 µL of SULFO-TAG Anti-ms cTnI Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-ms FABP3 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-ms Myl3 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-ms Troponin I (fast-twitch) Antibody

Custom Mulitplex

You may omit detection antibodies for any analyte not being measured. For one plate, combine 60 µL of each supplied detection antibody, then add Diluent 30 to bring the final volume to 3,000 µL.

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

Important: 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 precoated 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., prewetting) is required.

Protocol

Note: Follow Reagent Preparation before beginning this assay protocol.

STEP 1: Add Diluent 33 plus Additives

- ❑ Add 25 µL of Diluent 33 plus additives to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 30 minutes.

STEP 2: Add Sample or Calibrator

- ❑ Add 25 µL of the prepared Calibrator Standard or diluted samples to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well (~300 µL/well) of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well (~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.

Validation and Verification

MSD's validated testing is conducted following fit-for-purpose principles[9] and MSD design control procedures.

Validation. Bioanalytical and functional characterizations of calibrators, antibodies, and other assay components are completed to ensure the quality and consistency of reagents between lots. This includes plate coating uniformity, reagent, and component specificity testing for individual kit lots. Multiple control sample replicates in the specified matrices are tested to ensure the assay meets MSD's accuracy, precision, and sensitivity criteria.

Verification. Multiday analysis with multiple runs per day using 6–12 plates is performed as part of the release testing for each lot.

- Curve Fitting

  Calibration curve fitting methods, including weighting functions and 4- or 5-parameter logistic models, are evaluated on multiple runs to select the best curve-fitting algorithm.

- Sensitivity and Dynamic Range

  The lower limit of detection (LLOD) is established based on runs throughout assay development. It is a calculated concentration based on a signal 2.5 standard deviations above the average reading from the blank calibrators. This results in a signal that is significantly higher than the background. The dynamic (quantitative) range is established based on multiple runs from multiple lots. The limits of the range (the lower limit of quantification (LLOQ) and upper limit of quantification [ULOQ)] are the lowest and highest concentrations that can be measured with acceptable levels of precision and accuracy. The limits of quantification defined in this product insert are verified for each lot as part of the lot verification and quality control release.

- Accuracy and Precision

  Control samples made in the specified matrix are tested over multiple days to measure intrarun, interrun, and interlot accuracy and precision. Coefficient of variance (CV) information is presented in the product insert. During the validation process, the assay is tested over multiple days with multiple runs per day using a total of 15–20 complete kits. Precision and accuracy are verified for each lot as part of the lot verification and quality control release. The typical specification for precision is a CV of less than 20% for controls on both intra- and interday runs. The typical specification for accuracy includes a calculated concentration CV of less than 20%, accuracy within 20% of expected concentration, and a total error of less than 30%.

- Specificity, Matrix Effects, and Samples

  Assays are tested in the targeted matrix for nonspecific bindings. Spike recovery and dilution linearity are tested across the assay range to evaluate sample matrix effects. Normal samples for the specified species are tested to determine the normal range of biomarker concentration detected with the assay.

- Assay Robustness and Stability

  Freeze-thaw testing and accelerated stability studies for calibrators, antibodies, and controls are performed during assay development, and they are augmented with real-time stability studies on complete kits out to 24 months from the date of manufacture.

Representative data from the validation studies are presented in the following sections. The calibration curve and measured limits of detection for each lot can be found in the lot-specific COA that is included with each kit and available for download at www.mesoscale.com.

Analysis of Results

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the calibrators to a 4 parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analysis using a 5-parameter logistic model does not improve goodness-of-fit. Analyte concentrations were determined from the electrochemiluminescence signals by back-fitting to the calibration curve. These assays have a wide dynamic range (3–4 logs), which allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The calculations to establish calibration curves and determine concentrations were carried out using the MSD DISCOVERY WORKBENCH® analysis software.

The best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of two replicates at each calibrator level.

Typical Data

The following standard curves illustrate the dynamic range of the assay. Actual signals will vary. The best measurement of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards.

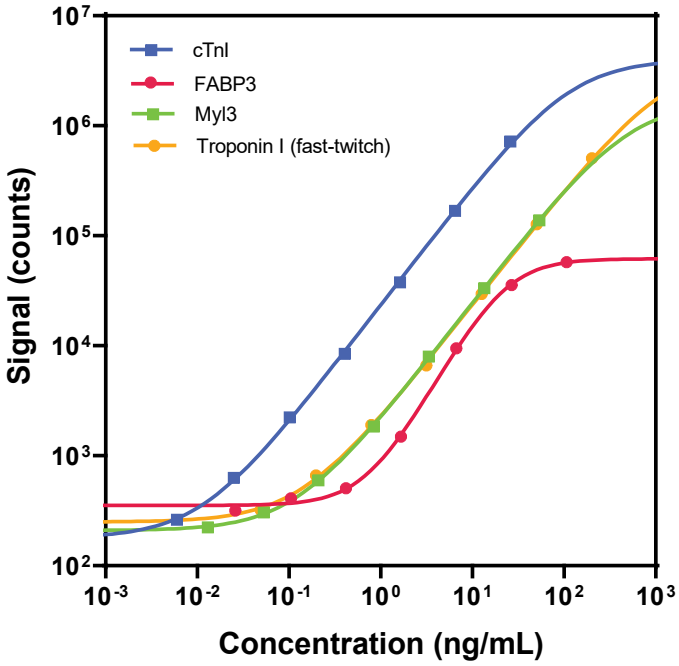

Figure 3. *Typical calibration curves.*

Sensitivity

The LLOD is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLOD shown below was calculated based on 28 runs.

The LLOQ is the lowest concentration at which the CV of the calculated concentration is <25% and the recovery of each analyte is within 75% to 125% of the known value. The quantitative range of the assay lies between the LLOQ and ULOQ.

The ULOQ is the highest concentration at which the CV of the calculated concentration is <20% and the recovery of each analyte is within 80% to 120% of the known value.

Table 5. LLOD, LLOQ, and ULOQ for each analyte in the Kit

	Median LLOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
cTnI	0.0088	0.07	20.0
FABP3	0.300	1.00	26.8
Myl3	0.0479	0.300	41.4
Troponin I (fast-twitch)	0.0846	0.505	155

Precision

Controls were made by spiking calibrator into mouse serum at three levels within the quantitative range of the assay. Analyte levels were measured using a minimum of 3 replicates on 41 runs over 14 days. Results are shown below.

Average intrarun %CV is the average %CV of the control replicates within an individual run. Interrun %CV is the variability of controls across 41 runs. Interlot %CV is the variability of controls across 2 kit lots.

Table 6. Intrarun and Interrun %CVs for each analyte in the Proinflammatory Panel 1 (rat) Kit

	Control	Runs	Average Conc. (ng/mL)	Average Intrarun %CV	Interrun %CV	Interlot %CV
cTnI	Control 1	41	11.0	6.1	8.4	4.8
	Control 2	41	1.58	4.7	8.6	5.6
	Control 3	41	0.430	4.2	9.3	6.7
FABP3	Control 1	41	12.6	6.8	10.9	10.0
	Control 2	41	3.25	6.0	9.3	7.9
	Control 3	41	1.25	6.2	12.9	12.5
Myl3	Control 1	41	26.9	5.1	8.0	3.7
	Control 2	41	6.98	5.3	7.1	1.8
	Control 3	41	0.489	5.2	10.0	7.9
Troponin I (fast-twitch)	Control 1	41	49.9	6.3	10.2	4.7
	Control 2	41	8.25	4.6	10.0	4.0
	Control 3	41	1.34	4.9	11.0	7.9

Dilution Linearity

To assess linearity, normal mouse serum, EDTA plasma, and heparin plasma samples from a commercial source were diluted 2-fold, 4-fold, 8-fold, and16-fold before testing. Percent recovery at each dilution was normalized to the dilution-adjusted 2-fold concentration. The average percent recovery shown below is based on samples within the quantitative range of the assay.

$$\%recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 7. Analyte percent recovery at various dilutions in each sample type

		cTnI		FABP3		Myl3		Troponin I (fast-twitch)	
Sample Type	Fold Dilution	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range
Serum (N=3)	2	100	—	100	—	100	—	100	—
	4	101	100–101	90	88–93	86	79–90	92	90–94
	8	100	96–104	95	93–99	95	94–96	100	100*
	16	102	93–113	100	90–110	90	87–92	—	—
EDTA Plasma (N=3)	2	100	—	100	—	100	—	100	—
	4	102	96–111	85	85*	85	83–89	105	104–107
	8	103	101–107	91	91*	94	92–96	96	96*
	16	101	96–107	97	87–109	92	92*	98	98*
Heparin Plasma (N=3)	2	100	—	100	—	100	—	100	–
	4	92	89–95	87	87*	86	85–87	96	94–98
	8	94	92–96	93	91–94	94	89–99	—	—
	16	101	97–106	94	88–103	—	—	—	—

dash (—) = not applicable

*A range of recovery cannot be provided since 2 of 3 samples were not within the quantitative range.

Note: Some assays showed significant matrix effects, which can be minimized by greater sample dilution

Spike Recovery

Normal mouse serum, EDTA plasma, and heparin plasma samples were diluted 4-fold and spiked with calibrators at multiple levels. The average percent recovery shown below is based on samples within the quantitative range of the assay.

$$\%recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 7. Analyte percent recovery at various dilutions in each sample type

	cTnI			FABP3		
Sample Type	Spike Conc. (ng/mL)	Average% Recovery	%Recovery Range	Spike Conc. (ng/mL)	Average% Recovery	%Recovery Range
Serum (N=6)	0.144–0.163	112	104–126	0.591–0.669	106	101–110
	0.578–0.650	114	105–123	2.37–2.68	115	106–120
	2.31–2.60	114	103–125	9.46–10.7	114	101–128
	0.144–0.163	117	101–138	0.591–0.669	99	88–109
EDTA Plasma (N=7)	0.578–0.650	118	99–132	2.37–2.68	103	91–116
	2.31–2.60	115	101–125	9.46–10.7	105	74–133
	0.144–0.163	106	103–110	0.591–0.669	105	100–110
	0.578–0.650	108	101–113	2.37–2.68	104	93–112
Heparin Plasma (N=7)	2.31–2.60	107	97–112	9.46–10.7	102	82–111
	0.144–0.163	112	104–126	0.591–0.669	106	101–110
	0.578–0.650	114	105–123	2.37–2.68	115	106–120
	2.31–2.60	114	103–125	9.46–10.7	114	101–128

Note: Some assays showed significant matrix effects, which can be minimized by greater sample dilution

	Myl3			Troponin I (fast-twitch)		
Sample Type	Spike Conc. (ng/mL)	Average% Recovery	%Recovery Range	Spike Conc. (ng/mL)	Average% Recovery	%Recovery Range
Serum (N=6)	0.336–0.346	121	104–137	1.08–1.26	103	90–110
	1.35–1.38	136	127–157	4.33–5.05	102	91–109
	5.38–5.53	140	126–163	17.3–20.2	97	89–102
	0.336–0.346	129	113–140	1.08–1.26	101	88–110
EDTA Plasma (N=7)	1.35–1.38	135	112–157	4.33–5.05	99	86–107
	5.38–5.53	132	124–140	17.3–20.2	92	81–101
	0.336–0.346	129	124–142	1.08–1.26	103	92–113
	1.35–1.38	142	132–150	4.33–5.05	97	85–106
Heparin Plasma (N=7)	5.38–5.53	138	121–154	17.3–20.2	89	76–96
	0.336–0.346	121	104–137	1.08–1.26	103	90–110
	1.35–1.38	136	127–157	4.33–5.05	102	91–109
	5.38–5.53	140	126–163	17.3–20.2	97	89–102

Note: Some assays showed significant matrix effects, which can be minimized by greater sample dilution

Specificity

To assess specificity of the individual assays, the Muscle Injury Panel 3 (mouse) was run using blended antibodies with individual calibrators (8.67 ng/mL cTnI; 35.7 ng/mL FABP3; 17.9 ng/mL Myl3; 67.3 ng/mL Troponin I (fast-twitch)). No significant nonspecific bindings were observed.

Stability

Kit components were tested for freeze-thaw stability. Results (not shown) demonstrated that blended calibrator can be refrozen and thawed 1 time without significantly affecting assay performance. Mouse serum and plasma samples can go through 5 freeze-thaw cycles without any significant changes in their measured concentrations.

Tested Samples

Serum, EDTA plasma, and heparin plasma samples were collected from normal CD-1 mice, diluted 4-fold, and tested with the Muscle Injury Panel 3 (mouse). The Medians and ranges of concentrations for each sample set are displayed below. Concentrations are corrected for sample dilution.

Table 5. Normal human samples tested in the Kit

Sample Type	Statistics	cTnI	FABP3	Myl3	Troponin I (fast-twitch)
Serum (N=37)	Median (ng/mL)	0.771	22.7	1.80	<LLOQ
	Range (ng/mL)	<LLOQ–5.68	<LLOQ–>ULOQ	<LLOQ–24.6	<LLOQ–11.1
	Number of Samples	28	28	28	28
	Samples in Quantitative Range	23	26	21	8
EDTA Plasma (N=17)	Median (ng/mL)	1.11	69.3	1.83	<LLOQ
	Range (ng/mL)	0.183–2.23	21.8–>ULOQ	0.652–20.3	<LLOQ–21.5
	Number of Samples	16	16	16	16
	Samples in Quantitative Range	16	11	16	3
Heparin Plasma (N=17)	Median (ng/mL)	3.04	98.1	2.04	<LLOQ
	Range (ng/mL)	0.855–5.32	42.3–>ULOQ	0.792–5.08	<LLOQ–4.06
	Number of Samples	16	16	16	16
	Samples in Quantitative Range	16	9	16	5

Assay Components

Calibrators

In the Muscle Injury Panel 3 (mouse) calibrator blend, mouse cTnI, mouse FABP3, and mouse Troponin I (fast-twitch) are native proteins. Full-length recombinant Myl3 protein was expressed in *E. coli*.

Antibodies

The antibody source species are described in Table 12.

Table 12. Antibody source species

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
cTnI	Mouse Monoclonal	Mouse Monoclonal
FABP3	Chicken Polyclonal	Chicken Polyclonal
Myl3	Mouse Monoclonal	Mouse Monoclonal
Troponin I (fast-twitch)	Mouse Monoclonal	Mouse Monoclonal

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3) Babuin L, Jaffe A S. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005 Nov 8;173(10):1191-02.
4) Gao Y, et al. Myosin light chain kinase as a multifunctional regulatory protein of smooth muscle contraction. IUBMB Life, 2001 Jun;51(6):337-44.
5) Kabaeva ZT, et al. Systematic analysis of the regulatory and essential myosin light chain genes: genetic variants and mutations in hypertrophic cardiomyopathy. Eur J Hum Genet. 2002 Nov;10(11):741-8.
6) Berna MJ, et al. Strategic use of immunoprecipitation and LC/MS/MS for trace-level protein quantification: myosin light chain 1, a biomarker of cardiac necrosis. Anal Chem. 2007 Jun 1;79(11):4199-205.
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8) Pritt ML, et al. Fabp3 as a biomarker of skeletal muscle toxicity in the rat: comparison with conventional biomarkers. Toxicol. Sci. 2008 Jun;103(2):382-96.

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Summary Protocol

Muscle Injury Panel 3 (mouse) Kit

MSD provides this summary protocol for your convenience.

Please read the entire detailed protocol before performing the

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Diluent 33 plus additives.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator blend 20-fold in Diluent 33 plus additives.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 4-fold in Diluent 33 plus additives before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each stock detection antibody 50-fold in Diluent 30.
- ❑ Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

STEP 1: Add Diluent 33 plus Additives

- ❑ Add 25 µL of Diluent 33 plus additives to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 30 minutes.

STEP 2: Add Sample or Calibrator

- ❑ Add 25 µL of the prepared Calibrator Standard or diluted samples to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well (~300 µL/well) of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well (~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.

Plate Diagram

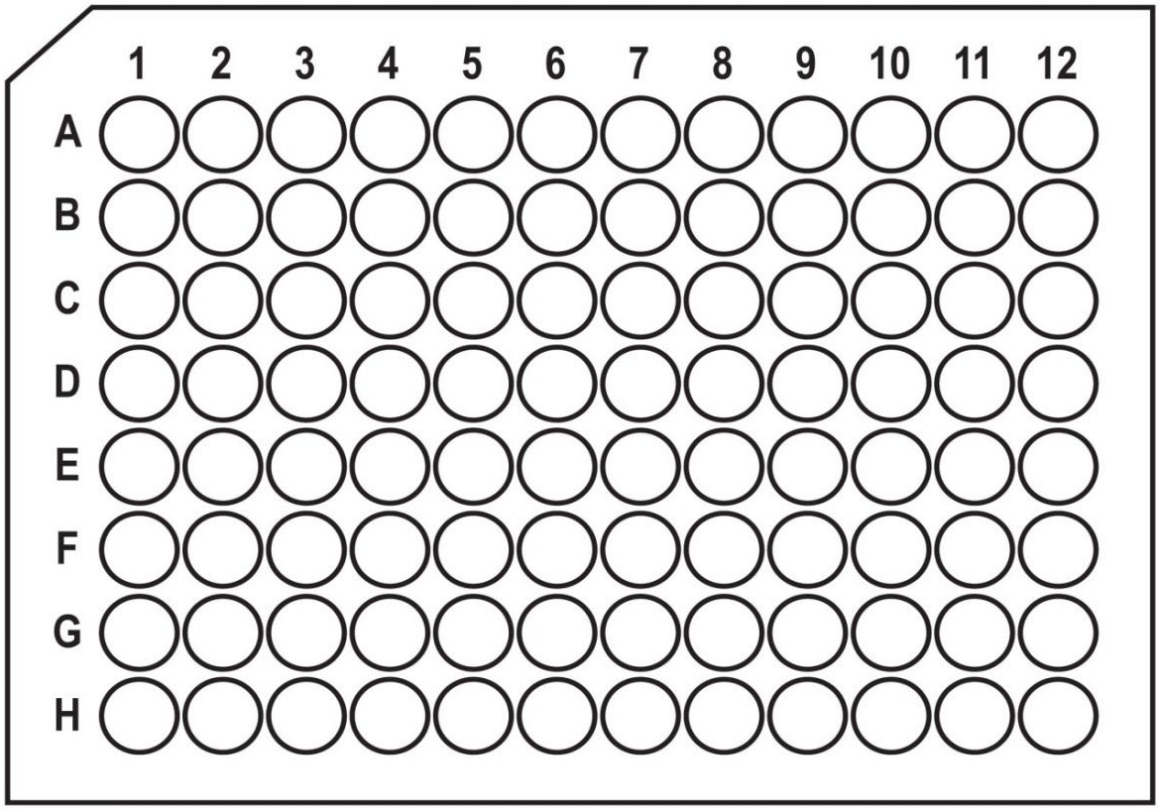

Figure 7. Plate diagram; a similar plate layout can be created in Excel and easily imported into DISCOVERY WORKBENCH software.