Muscle Injury Panel Kits

cTnI, cTnT, FABP3, Myl3, and sTnI

	Catalog No.
Muscle Injury Panel 1 (rat) Kit	K15181C
Cardiac Injury Panel 2 (rat) Kit	K15155C
Cardiac Injury Panel 3 (rat) Kit	K15161C

MSD®

MSD Toxicology Assays

Muscle Injury Panel 1 (rat) Kit
cTnI, cTnT, FABP3, Myl3, sTnI

Cardiac Injury Panel 2 (rat) Kit
cTnI, cTnT, FABP3

Cardiac Injury Panel 3 (rat) Kit
cTnI, cTnT, FABP3, Myl3

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.

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.
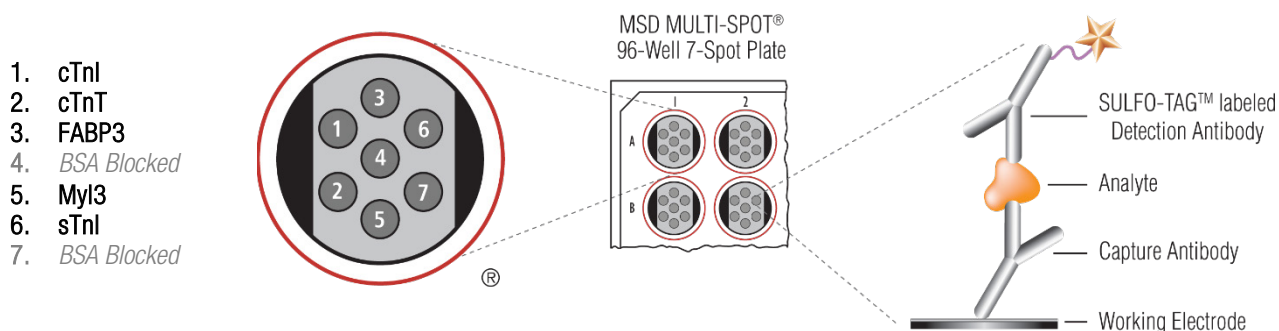
When muscle tissue is damaged, the troponin-tropomyosin complex breaks down and troponin I and troponin T are released into the blood. Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) can be readily distinguished from their skeletal muscle analogs (sTnT and sTnI), allowing confirmation of cardiac muscle tissue damage over skeletal muscle tissue damage. Troponins are excellent biomarkers for research on myocardial injury in cardiotoxicity because of the demonstrated tissue-specificity of cardiac and skeletal troponins.[2]

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 myosin to its cargo. Two heavy chain subunits intertwine to form the head and tail domains. 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)—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 during research 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.

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Principle of the Assay

MSD toxicology assays provide rapid and convenient methods for measuring the levels of protein targets within a single, small-volume sample. The assays in the Muscle Injury Panel 1 (rat) 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 MSD SULFO-TAG™ labels throughout one or more incubation periods. Analytes in the sample 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.

1. cTnI
2. cTnT
3. FABP3
4. *BSA Blocked*
5. MyI3
6. sTnI
7. *BSA Blocked*

MSD MULTI-SPOT®
96-Well 7-Spot Plate

SULFO-TAG™ labeled Detection Antibody

Analyte

Capture Antibody

Working Electrode

Figure 1. Spot diagram showing the 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.

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Kit Components

Table 1. Reagents supplied with all kits

Product Description	Storage	Catalog No.	Size	Quantity per Kit		
				1 Plate	5 Plates	25 Plates
MULTI-SPOT® 96-Well Muscle Injury Panel 1 (rat) Plate	2–8 °C	N75181A-1	7-spot	1 plate	5 plates	25 plates
Muscle Injury Panel 1 (rat) Calibrator Blend (20X)	≤-70 °C	C0181-2	20 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	—	1 mL	1 vial	1 vial	5 vials
0.5 M EDTA pH 8.0	RT	—	4 mL	1 bottle	1 bottle	5 bottles
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles

RT = room temperature
Dash (—) = not applicable

Table 2. Components that are supplied with specific kits

Product Description	Storage	Catalog No.	Size	Quantity per Kit		
				1 Plate	5 Plates	25 Plates
SULFO-TAG Anti-rat cTnI Antibody (50X)	2–8 °C	D23HR-2	75 µL	1 vial	—	—
		D23HR-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-rat cTnT Antibody (50X)	2–8 °C	D23EF-2	75 µL	1 vial	—	—
		D23EF-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-rat FABP3 Antibody (50X)	2–8 °C	D23HT-2	75 µL	1 vial	—	—
		D23HT-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-rat Myl3 Antibody (50X)	2–8 °C	D23JI-2	75 µL	1 vial	—	—
		D23JI-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-rat sTnI Antibody (50X)	2–8 °C	D23IM-2	75 µL	1 vial	—	—
		D23IM-3	375 µL	—	1 vial	5 vials

Dash (—) = not applicable

Additional Materials and Equipment—not supplied

- ❑ 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
- ❑ MSD Wash Buffer (Catalog No. R61AA-1)
- ❑ Liquid handling equipment for the 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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

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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. Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.

- 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 before 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 interplate 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 the 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.

Table 3. EDTA and DDT additives

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 Calibrator blend for the Muscle Injury Panels is supplied at a 20-fold higher concentration than the recommended highest Calibrator. For each assay, a 7-point standard curve is recommended with 3-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. 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:

- ❑ Prepare the highest standard by adding 12 µL of stock Calibrator to 228 µL of Diluent 7 + Additives. Mix well.
- ❑ Prepare the next standard by transferring 80 µL of the highest standard to 160 µL of Diluent 7 + Additives. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
- ❑ 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 before addition to the plate.

Dilute Samples

Rat 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. Depending on the sample set under investigation, higher or lower dilution factors may be necessary. If needed, additional Diluent 7 can be purchased at www.mesoscale.com.

Diluted samples should be incubated at room temperature without shaking for 30 minutes before adding 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 of Muscle Injury Panel 1 (rat), 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
- ❑ 60 μL of 50X SULFO-TAG Anti-rat Myl3 Antibody
- ❑ 60 μL of 50X SULFO-TAG Anti-rat sTnI Antibody
- ❑ 2,700 μL of Diluent 30

For one plate of Cardiac Injury Panel 3 (rat), 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
- ❑ 60 μL of 50X SULFO-TAG Anti-rat Myl3 Antibody
- ❑ 2,760 μL of Diluent 30

For one plate of Cardiac Injury Panel 2 (rat), 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

Prepare Wash Buffer

MSD provides Wash Buffer (Catalog No. R61AA-1) 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.

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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 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: Complete the steps described in **Reagent Preparation** (above) 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 1: Step 2: Add Sample or Calibrator

❑ Add 25 µL of sample or Calibrator (which has been preincubated 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

Note: 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.

Note: 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 analytes 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.

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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, 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 $1/Y^2$ 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 the 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 interday %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 multiday (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 and data illustrate the dynamic range of the assay. Actual signals will vary. The 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.
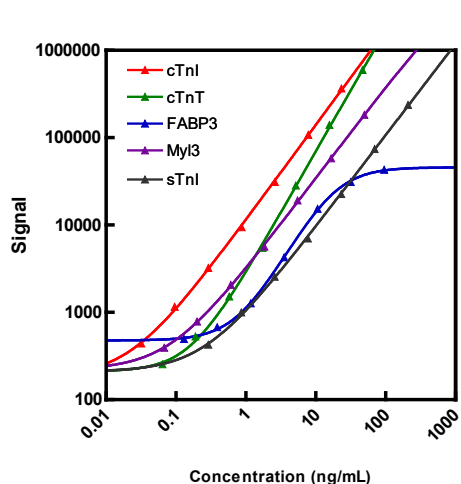
cTnI		
Conc. (ng/mL)	Average Signal	%CV
0	107	12.4
0.0321	440	6.2
0.0963	1,155	7.0
0.289	3,214	6.4
0.867	9,433	5.5
2.60	31,018	4.5
7.80	107,673	4.0
23.4	365,820	7.0

cTnT		
Conc. (ng/mL)	Average Signal	%CV
0	120	8.5
0.0645	254	6.8
0.193	529	6.7
0.580	1,519	5.6
1.74	5,468	5.1
5.22	28,325	4.1
15.7	139,526	5.9
47.0	594,838	5.6

FABP3		
Conc. (ng/mL)	Average Signal	%CV
0	421	6.8
0.131	496	7.4
0.392	675	4.9
1.18	1,270	7.1
3.53	4,257	5.2
10.6	15,362	5.7
31.7	31,031	6.0
95.2	42,881	6.4

Myl3		
Conc. (ng/mL)	Average Signal	%CV
0	167	10.8
0.0682	394	4.8
0.205	785	6.6
0.614	2,066	5.1
1.84	5,645	6.4
5.52	19,059	3.9
16.6	58,134	3.0
49.7	182,582	3.5

sTnI		
Conc. (ng/mL)	Average Signal	%CV
0	165	9.6
0.286	427	5.8
0.858	996	3.7
2.58	2,556	3.0
7.74	7,019	3.8
23.2	22,614	3.5
69.7	74,092	4.5
209	237,149	6.1

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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 multiplate, multiday 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 and Myl3, the specification on percent recovery was widened to 75% to 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% to 125%.

Table 4. LLOD, LLOQ, and ULOQ for each analyte in the Muscle Injury Panel (rat) Kits

	cTnI	cTnT	FABP3	Myl3	sTnI
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
LLOD	0.00400	0.0171	0.155	0.0865	0.0472
LLOQ	0.0977	0.488	0.391	0.170	0.781
ULOQ	20.0	35.0	15.0	44.0	160

Precision

Control samples of high, mid, and low levels of each analyte were measured 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 controls were run in triplicate or quadruplicate on each of 9 plates run across 3 days.

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

Interplate %CV is the variability of controls across 9 plates over 3 days.

Table 5. Intraplate and Interplate %CVs for each analyte in the Muscle Injury Panel (rat) Kits

	Control	Plates	Average Conc. (ng/mL)	Average Intraplate %CV	Interplate %CV
cTnI	High	9	9.36	3.8	5.9
	Mid	9	1.31	3.9	6.0
	Low	9	0.278	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
FAPB3	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	0.319	6.0	7.1
sTnI	High	9	108	4.3	5.8
	Mid	9	17.4	2.4	3.1
	Low	9	2.88	3.5	10.5

Dilution Linearity

To assess linearity, serum, EDTA plasma and heparin plasma samples from Sprague-Dawley rats were diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. All samples were taken from normal rats. The concentrations shown below have been corrected for dilution (concentration = measured × 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).

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

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Table 6: Dilution Linearity: cTnI and cTnT

Sample	Fold Dilution	cTnI			cTnT		
		Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum No. 1	2	19.4	0.2	—	13.2	2.9	—
	4	17.3	3.8	89	12.5	1.4	95
	8	16.8	7.1	97	12.4	2.2	99
	16	15.7	6.2	94	11.4	5.1	93
Serum No. 2	2	5.82	7.6	—	3.7	11.0	—
	4	5.99	4.2	103	3.3	1.8	89
	8	6.32	1.4	105	< LLOQ	—	—
	16	5.72	2.4	91	< LLOQ	—	—
Serum No. 3	2	< LLOQ	—	—	< LLOQ	—	—
	4	< LLOQ	—	—	< LLOQ	—	—
	8	< LLOQ	—	—	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
EDTA Plasma No. 1	2	< LLOQ	—	—	< LLOQ	—	—
	4	< LLOQ	—	—	< LLOQ	—	—
	8	< LLOQ	—	—	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
EDTA Plasma No. 2	2	< LLOQ	—	—	< LLOQ	—	—
	4	< LLOQ	—	—	< LLOQ	—	—
	8	< LLOQ	—	—	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
EDTA Plasma No. 3	2	< LLOQ	—	—	< LLOQ	—	—
	4	< LLOQ	—	—	< LLOQ	—	—
	8	< LLOQ	—	—	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
Heparin Plasma No. 1	2	1.62	2.0	—	< LLOQ	—	—
	4	1.57	2.8	97	< LLOQ	—	—
	8	1.52	2.6	97	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
Heparin Plasma No. 2	2	1.30	4.2	—	< LLOQ	—	—
	4	1.25	5.1	96	< LLOQ	—	—
	8	1.29	1.2	103	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
Heparin Plasma No. 3	2	1.30	5.0	—	< LLOQ	—	—
	4	1.27	5.7	98	< LLOQ	—	—
	8	1.24	2.7	97	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—

LLOQ = lower limit of quantification
Dash (–) = not applicable

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Table 7. Dilution Linearity: FABP3 and Myl3

Sample	Fold Dilution	FABP3			Myl3		
		Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum No. 1	2	> ULOQ	—	—	15.5	5.5	—
	4	> ULOQ	—	—	13.4	0.5	86
	8	> ULOQ	—	—	13.8	4.6	103
	16	> ULOQ	—	—	12.9	2.2	93
Serum No. 2	2	> ULOQ	—	—	8.32	11.3	—
	4	> ULOQ	—	—	7.53	2.5	90
	8	> ULOQ	—	—	7.23	3.5	96
	16	124	10.9	—	6.57	4.7	91
Serum No. 3	2	3.24	6.0	—	0.348	7.0	—
	4	3.05	3.7	94	0.306	5.1	88
	8	3.28	6.3	108	< LLOQ	—	—
	16	< LLOQ	—	—	< LLOQ	—	—
EDTA Plasma No. 1	2	> ULOQ	—	—	1.93	1.5	—
	4	54.7	10.4	111	1.88	3.5	98
	8	55.6	3.7	102	1.87	3.9	99
	16	52.1	6.5	94	1.47	5.1	79
EDTA Plasma No. 2	2	> ULOQ	—	—	2.94	7.2	—
	4	40.8	13.8	100	2.67	3.5	91
	8	38.1	10.3	93	2.56	6.2	96
	16	37.7	4.4	99	1.91	8.9	74
EDTA Plasma No. 3	2	6.00	2.8	—	0.538	8.5	—
	4	5.47	5.1	91	0.478	8.5	89
	8	5.29	6.5	97	0.507	12.4	106
	16	< LLOQ	—	—	< LLOQ	—	—
Heparin Plasma No. 1	2	> ULOQ	—	—	3.08	3.7	—
	4	> ULOQ	—	—	2.84	3.3	92
	8	66.8	6.3	—	2.58	8.9	91
	16	67.2	7.2	101	2.10	8.3	82
Heparin Plasma No. 2	2	> ULOQ	—	—	3.43	3.1	—
	4	56.1	5.4	—	3.00	4.4	87
	8	55.7	1.0	99	2.90	5.9	97
	16	50.6	6.5	91	2.48	8.8	86
Heparin Plasma No. 3	2	> ULOQ	—	—	3.48	5.0	—
	4	59.4	3.2	—	3.12	3.1	90
	8	57.9	2.9	98	3.10	3.4	100
	16	51.8	1.6	89	2.60	0.4	84

ULOQ = upper limit of quantification
LLOQ = lower limit of quantification
Dash (–) = not applicable

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Table 8. Dilution Linearity: sTnI

Sample	Fold Dilution	sTnI Concentration (ng/mL)	Concentration %CV	Percent Recovery
Serum No. 1	2	23.1	3.4	—
	4	22.4	5.4	97
	8	22.0	3.9	98
	16	21.5	3.3	98
Serum No. 2	2	14.9	7.8	—
	4	14.2	3.7	95
	8	13.9	6.1	98
	16	13.1	1.1	95
Serum No. 3	2	< LLOQ	—	—
	4	< LLOQ	—	—
	8	< LLOQ	—	—
	16	< LLOQ	—	—
EDTA Plasma No. 1	2	20.6	6.1	—
	4	20.4	2.8	99
	8	19.5	6.8	95
	16	19.3	9.0	99
EDTA Plasma No. 2	2	13.0	11.7	—
	4	13.3	6.5	102
	8	13.1	7.5	98
	16	< LLOQ	—	—
EDTA Plasma No. 3	2	< LLOQ	—	—
	4	< LLOQ	—	—
	8	< LLOQ	—	—
	16	< LLOQ	—	—
Heparin Plasma No. 1	2	16.4	2.7	—
	4	16.0	2.6	97
	8	15.3	2.4	96
	16	15.3	6.4	100
Heparin Plasma No. 2	2	14.1	8.1	—
	4	13.8	2.3	98
	8	13.3	2.3	97
	16	< LLOQ	—	—
Heparin Plasma No. 3	2	13.9	3.9	—
	4	14.0	5.8	101
	8	13.7	2.0	98
	16	12.7	4.9	93

LLOQ = lower limit of quantification
Dash (–) = not applicable

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 are outside of the range of quantitation.

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

Table 9. Spike recovery: cTnI and cTnT

Sample	cTnI				cTnT			
	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 #1	0	1.50	2.6	—	0	0.986	5.2	—
	0.260	1.80	5.2	102	0.522	1.55	2.4	103
	0.780	2.37	3.8	104	1.57	2.53	5.4	99
	2.34	3.90	8.3	102	4.70	5.84	3.8	103
Serum #2	0	0	—	—	0	0	—	—
	0.260	0.323	2.5	124	0.522	0.396	4.3	76
	0.780	0.925	3.9	119	1.57	1.27	3.8	81
	2.34	2.67	1.9	114	4.70	4.20	2.1	89
Serum #3	0	0	—	—	0	0	—	—
	0.260	0.332	7.0	128	0.522	0.443	6.0	85
	0.780	0.982	2.3	126	1.57	1.37	0.9	88
	2.34	2.80	5.6	120	4.70	4.31	4.0	92
EDTA Plasma #1	0	0.0388	1.5	—	0	0	—	—
	0.260	0.374	4.0	125	0.522	0.459	3.7	88
	0.780	1.04	2.6	127	1.57	1.44	1.2	92
	2.34	2.74	3.3	115	4.70	4.56	1.9	97
EDTA Plasma #2	0	0.0262	5.1	—	0	0	—	—
	0.260	0.373	5.5	130	0.522	0.457	8.8	87
	0.780	0.975	4.1	121	1.57	1.35	1.0	86
	2.34	2.74	3.7	116	4.70	4.42	3.1	94
EDTA Plasma #3	0	0	—	—	0	0	—	—
	0.260	0.350	1.1	135	0.522	0.443	5.7	85
	0.780	1.00	2.0	128	1.57	1.34	1.1	86
	2.34	2.77	2.4	118	4.70	4.32	0.7	92
Heparin Plasma #1	0	0.269	1.8	—	0	0.0485	35.0	—
	0.260	0.590	5.9	111	0.522	0.577	3.6	101
	0.780	1.20	3.9	114	1.57	1.54	3.1	95
	2.34	2.99	3.6	115	4.70	4.70	3.3	99
Heparin Plasma #2	0	0.276	6.4	—	0	0.0576	86.7	—
	0.260	0.611	3.2	114	0.522	0.588	3.7	101
	0.780	1.27	0.2	120	1.57	1.61	0.9	99
	2.34	2.82	8.4	108	4.70	4.40	9.4	92
Heparin Plasma #3	0	0.325	3.1	—	0	0.133	10.4	—
	0.260	0.612	2.2	105	0.522	0.665	2.2	102
	0.780	1.10	1.4	100	1.57	1.58	3.9	93
	2.34	2.71	2.2	102	4.70	4.84	3.7	100

Dash (—) = not applicable

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Table 10. Spike recovery: FABP3 and Myl3

Sample	FABP3				Myl3			
	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 #1	0	28.4	10.1	—	0	1.51	4.2	—
	1.06	26.9	7.6	91	0.552	2.28	3.5	111
	3.17	30.6	8.8	97	1.66	3.47	4.2	110
	9.52	32.0	21.7	84	4.97	6.81	6.1	105
Serum #2	0	0.643	4.8	—	0	0.0492	40.1	—
	1.06	1.84	2.2	108	0.552	0.692	6.9	115
	3.17	4.51	4.6	118	1.66	2.05	5.2	120
	9.52	12.1	10.8	119	4.97	6.14	6.5	122
Serum #3	0	1.05	10.2	—	0	0.0557	8.5	—
	1.06	2.19	4.8	104	0.552	0.734	4.4	121
	3.17	4.86	6.1	115	1.66	2.06	4.3	120
	9.52	12.6	2.3	119	4.97	6.14	4.4	122
EDTA Plasma #1	0	11.3	5.9	—	0	0.395	2.9	—
	1.06	12.5	11.4	101	0.552	1.08	6.4	114
	3.17	14.2	1.2	98	1.66	2.39	0.6	116
	9.52	19.0	2.5	91	4.97	6.29	2.5	117
EDTA Plasma #2	0	8.66	4.6	—	0	0.493	6.2	—
	1.06	10.1	6.6	104	0.552	1.15	8.1	110
	3.17	12.7	4.1	107	1.66	2.46	3.2	114
	9.52	17.4	5.6	96	4.97	6.21	3.6	114
EDTA Plasma #3	0	1.33	8.3	—	0	0.0813	19.2	—
	1.06	2.51	4.0	105	0.552	0.772	5.6	122
	3.17	5.15	1.1	114	1.66	2.16	2.6	124
	9.52	12.1	6.5	111	4.97	6.28	3.6	124
Heparin Plasma #1	0	12.1	7.4	—	1.92	0.516	11.4	—
	1.06	13.8	4.6	105	4.50	1.24	11.3	116
	3.17	16.9	12.0	110	9.22	2.53	7.9	116
	9.52	22.4	8.0	103	23.5	6.28	3.7	115
Heparin Plasma #2	0	13.1	30.7	—	1.98	0.535	5.5	—
	1.06	12.4	4.1	88	4.48	1.24	1.5	114
	3.17	14.8	6.9	91	9.42	2.58	3.6	118
	9.52	20.6	8.3	91	24.3	6.48	2.8	118
Heparin Plasma #3	0	12.8	0.9	—	0	0.671	3.4	—
	1.06	14.6	8.4	106	0.552	1.43	3.2	117
	3.17	16.4	1.2	103	1.66	2.66	4.3	114
	9.52	23.8	9.2	107	4.97	6.69	0.5	119

Dash (—) = not applicable

Table 11. Spike recovery: sTnI

Sample	sTnI			
	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum #1	0	3.50	1.6	—
	2.32	6.14	2.8	106
	6.95	11.0	3.2	105
	20.9	26.1	3.2	107
Serum #2	0	0.0288	64.0	—
	2.32	2.48	5.3	106
	6.95	7.20	5.9	103
	20.9	20.6	4.3	98
Serum #3	0	0.0136	71.4	
	2.32	2.44	1.5	105
	6.95	7.25	2.4	104
	20.9	21.7	3.6	104
EDTA Plasma #1	0	4.40	2.7	—
	2.32	6.92	6.1	103
	6.95	12.0	5.1	106
	20.9	25.4	2.5	101
EDTA Plasma #2	0	3.19	5.6	—
	2.32	5.79	4.1	105
	6.95	11.1	1.7	109
	20.9	24.3	3.3	101
EDTA Plasma #3	0	0.0838	63.1	—
	2.32	2.59	3.0	108
	6.95	7.26	1.5	103
	20.9	20.2	3.1	96
Heparin Plasma #1	0	3.23	8.2	—
	2.32	5.70	7.6	103
	6.95	10.8	5.4	106
	20.9	24.3	6.2	101
Heparin Plasma #2	0	3.00	3.3	—
	2.32	5.72	3.0	108
	6.95	11.3	6.2	114
	20.9	26.0	5.4	109
Heparin Plasma #3	0	3.28	0.4	—
	2.32	5.79	3.5	103
	6.95	10.2	3.3	100
	20.9	24.3	2.4	101

Dash (—) = not applicable

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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.

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. 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, and approximately 200-fold less Myl3 was measured in fast-twitch.

Table 12. Muscle homogenate samples tested for assay specificity

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	1,000	22.6	1,000	25.1	10,000	125.2	1,000	5.00	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	1000	12.2	100	0.0837	1,000	40.9
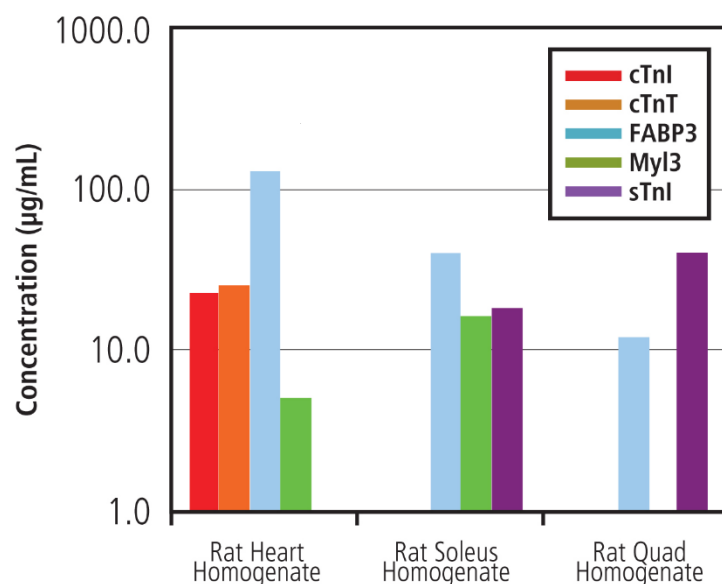
Figure 2. Muscle homogenate samples tested for assay specificity.

Specificity of the Assays for Individual Calibrators:

The Muscle Injury Panel 1 assay was run with each single Calibrator at a high level 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 nonspecific signal to the specific signal.

Table 13. Assay specificity for individual calibrators

Spot	Blended Detection Antibodies and Single Calibrator % Cross-Reactivity				
	cTnI	cTnT	FABP3	Myl3	sTnI
cTnI	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
Myl3	< 0.1	< 0.1	< 0.1	100	< 0.1
sTnI	< 0.1	< 0.1	< 0.1	< 0.1	100

Specificity of the Detection Antibodies:

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 cTnI Calibrator on the cTnI spot is reported out by the cTnI, cTnT, and sTnI detection antibodies.

Table 14. Detection antibody specificity

Spot	Blended Calibrator and Single Detection Antibody % Cross-Reactivity				
	cTnI	cTnT	FABP3	Myl3	sTnI
cTnI	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
Myl3	< 0.1	< 0.1	< 0.1	100	0.11
sTnI	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.

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Tested Samples

Serum, EDTA plasma, and heparin 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.

Table 15. Samples tested in the Muscle Injury Panel (rat) Kits

Sample	Statistic	cTnI	cTnT	FABP3	Myl3	sTnI
Serum	Median (ng/mL)	1.59	0.83	19.84	0.61	< 1.56
	Range (ng/mL)	0.436–3.53	< 0.976–2.04	5.28–> 30.0	0.25–1.05	< 1.56
	N	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	0.432–1.17	< 1.56
	N	10	10	10	10	10
Heparin Plasma	Median (ng/mL)	1.81	1.06	28.35	0.55	< 1.56
	Range (ng/mL)	0.372–4.10	< 0.976–2.79	4.12–> 30.0	0.162–1.16	< 1.56
	N	10	10	10	10	10

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Assay Components

Calibrators

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.

Antibodies

Table 16. Antibody source species

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

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. doi: 10.1007/s11095-005-9045-3.
2. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005 Nov 8;173(10):1191-202. doi: 10.1503/cmaj/051291.

Summary Protocol

Muscle Injury Panel Kits

MSD provides this summary protocol for your convenience.

Please read the entire detailed protocol before performing the Muscle Injury Panel 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 concentrations in Diluent 7.

❑ Prepare 8 calibration solutions using the supplied Calibrator:

- Dilute the stock Calibrator 20-fold in Diluent 7 + Additives.

- Perform a series of 3-fold dilution steps and prepare a zero Calibrator.

❑ Dilute serum and plasma samples 4-fold in Diluent 7 + Additives before adding to the plate.

❑ Incubate the diluted Calibrators and diluted samples at room temperature for 30 minutes before adding them 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 150 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 150 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.

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Plate Diagrams

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