

Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Rat Skeletal Troponin I (sTnI) Assay Kit

1-Plate Kit

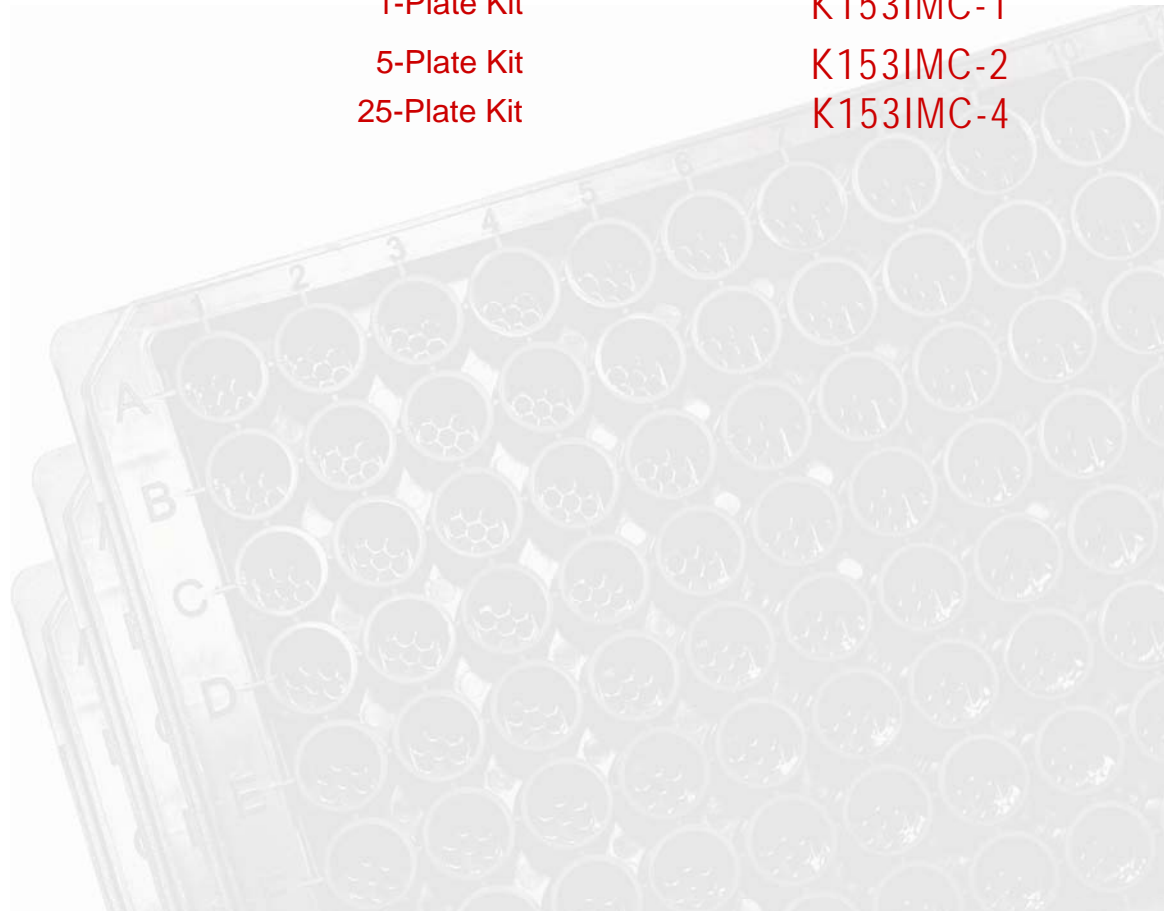
K153IMC-1

5-Plate Kit

K153IMC-2

25-Plate Kit

K153IMC-4



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MSD Toxicology Assays

Rat Skeletal Troponin I Assay Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

Ordering information

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Introduction

introduction

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

The three subunits of troponin are:

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

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

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 Rat Skeletal Troponin I (sTnI) Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibody for sTnI. The user adds the sample and a solution containing the labeled detection antibody—anti-sTnI labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Skeletal Troponin I in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte 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 sTnI present in the sample.

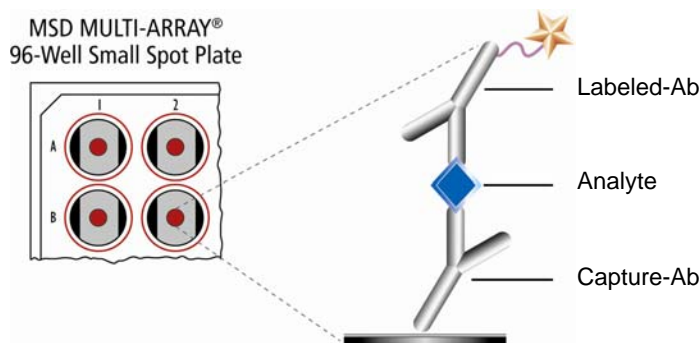


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K153IMC-1	K153IMC-2	K153IMC-4
Small-SPOT 96-well Rat Skeletal Troponin I Plate	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-rat sTnI Antibody (50X) ¹	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat Skeletal Troponin I Calibrator (20X) sTnI: 4 µg/mL	≤ -70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 7 R54BB-4 (5 mL) R54BB-3 (50 mL)	≤ -10°C	2 bottles (5 mL ea)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 30 R50AB-4 (25 mL)	≤ -10°C	1 bottle (25 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
25 mM DTT	≤ -10°C	1 vial (1 mL)	1 vial (1 mL)	5 vials (1 mL)
0.5 M EDTA pH 8.0	RT	1 bottle (4 mL)	1 bottle (4 mL)	5 bottles (4 mL)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)



Required Materials and Equipment - not supplied

required materials and equipment — not supplied

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

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

V Safety

s a f e t y

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

VI Reagent Preparation

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

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

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

Prepare Assay Diluent GF1 + Additives

For the Rat Skeletal Troponin I Assay, samples and calibrators are diluted in Diluent 7 that contains EDTA and DTT. These two additives must be added into the diluent by the user before each assay is carried out. EDTA and DTT additive stocks are provided at the concentrations in the table below.

For one plate combine:

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

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

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

Prepare Calibrator and Control Solutions

Calibrators for the Rat Skeletal Troponin I Assay 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 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	sTnI (pg/mL)	Dilution Factor
Stock Cal. Vial	4000000	
STD-01	200000	20
STD-02	66667	3
STD-03	22222	3
STD-04	7407	3
STD-05	2469	3
STD-06	823	3
STD-07	274	3
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

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

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

Dilution of Samples (optional)

For serum and plasma from normal rats, no dilution is necessary. However, for rats that have suffered a muscle injury, 2–20X dilution of serum or plasma samples is sometimes 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 Antibody is provided at 50X stock of Anti-rat sTnI Antibody. The working Detection Antibody Solution should contain 1X as final concentration. For each plate used, dilute 60 μ L of the stock Detection Antibody stock into a final volume of 3 mL 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 analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

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

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

Notes

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

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

VIII Analysis of Results

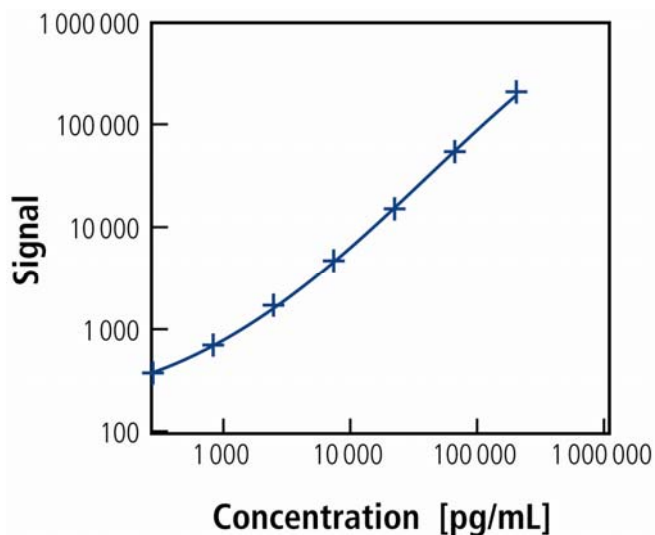
analysis of results

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

IX Typical Standard Curve

typical standard curve

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



Conc. (pg/mL)	Mean	%CV
0	213	1.8
274	358	2.9
823	704	4.1
2469	1672	8.5
7407	4568	7.9
22222	14847	7.8
66667	53441	6.9
200000	206052	3.4

X Sensitivity

sensitivity

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

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

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

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%.

	sTnl (pg/mL)
LLOD	374
LLOQ	781
ULOQ	160000

XI Precision

precision

High, mid, and low control samples were measured on 21 plates across 7 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. Controls are run neat. The average intra-plate %CV and inter-plate %CV of the concentrations are shown below.

	Control	Plates	Average Conc. (pg/mL)	Intra-Plate	Inter-Plate
				Average %CV	%CV
sTnl	High	21	100445	3.6	6.2
	Mid	21	15824	3.6	5.6
	Low	21	2797	4.5	7.2

XII Spike Recovery

spike recovery

Individual, normal rat serum, heparin and EDTA plasma samples were spiked with the Rat Skeletal Troponin I Calibrator at multiple concentrations throughout the range of the assay. The samples were diluted 2-fold and then spiked with Rat Skeletal Troponin I Calibrator at the levels indicated in the below table.

% Recovery = measured / expected x 100

Sample	Spike Level (pg/mL)	Conc. (pg/mL)	Conc. %CV	% Recovery
Spiked Serum	20000	21184	4.0	108
	6667	7220	4.6	106
	2222	2506	3.7	106
	0	<LLOD	-	-
Spiked EDTA Plasma	20000	21703	2.1	100
	6667	6718	3.7	96
	2222	2186	5.3	91
	0	<LLOD		
Spiked Heparin Plasma	20000	21797	3.8	109
	6667	7139	3.9	101
	2222	2414	3.0	96
	0	<LLOD	-	-

XIII Linearity

linearity

To assess linearity, rat serum, EDTA and heparin plasma samples were spiked with the Rat Skeletal Troponin I Calibrator and further diluted 10-fold, 50-fold, and 250-fold. 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 measured from the previous dilution (expected).

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

Sample	Fold Dilution	Conc. (pg/mL)	Conc. %CV	% Recovery
Serum	Spiked	90222	4.0	
	10	81150	3.5	90
	50	82650	5.4	102
	250	61000	13.0	74
EDTA Plasma	Spiked	83170	3.9	
	10	74270	4.7	89
	50	74100	6.5	100
	250	53000	11.7	71
Heparin Plasma	Spiked	84358	5.8	
	10	74820	6.3	87
	50	75350	5.5	101
	250	54250	13.9	70

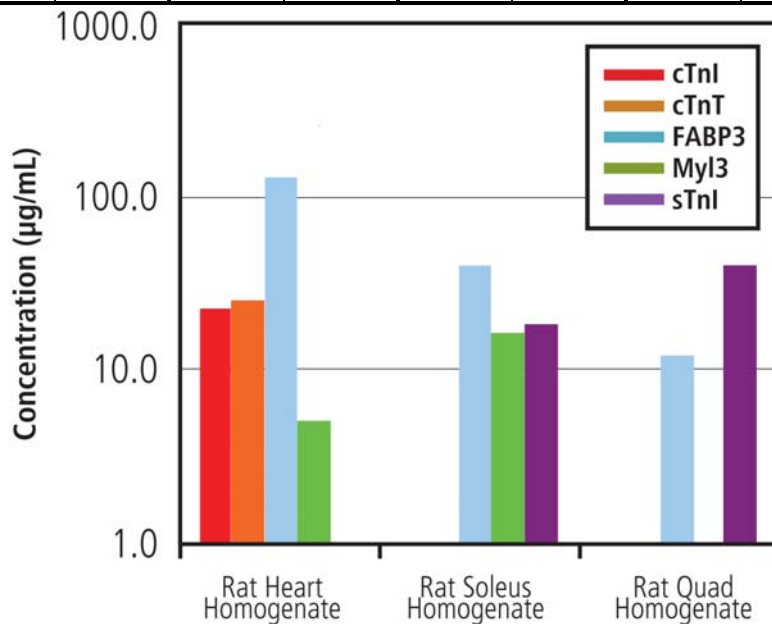
XIV Specificity

specificity

Specificity of sTnI was demonstrated by testing rat muscle homogenates on MIP-1 panel by running the assay with single Calibrators and single detection antibodies. Rat sTnI is the same assay used for the MSD Muscle Injury Panel 1 panel.

Tissue homogenates from heart, fast twitch, and slow twitch muscle were tested at 100X, 1000X and 10000X sample dilution. The assay for skeletal Troponin I was specific for fast and slow twitch skeletal muscle.

Sample Group	cTnI		cTnT		FABP3		MyI3		Skeletal TnI	
	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)	Sample Dilution	Conc. (µg/mL)
Rat Heart Homogenate	1000	22.6	1000	25.1	10000	125.2	1000	5.0	100	< LLOD
Rat Soleus Homogenate (slow twitch)	100	< LLOD	100	< LLOD	10000	38.8	1000	16.4	1000	18.1
Rat Quad Homogenate (fast twitch)	100	< LLOD	100	< LLOD	1000	12.2	100	0.08	1000	40.9



XV Samples

s a m p l e s

Serum, heparin plasma, and EDTA plasma samples collected from normal Sprague-Dawley rats were measured neat on the rat sTnl assay. Shown below are the median and range of concentrations for each sample set. Median levels of Skeletal Troponin I was below the quantitative range for all samples.

		sTnl
Serum	Median (ng/mL)	336
	Range (ng/mL)	102 - 1878
	# of samples	21
EDTA Plasma	Median (ng/mL)	378
	Range (ng/mL)	106 - 936
	# of samples	10
Heparin Plasma	Median (ng/mL)	27
	Range (ng/mL)	0 - 514
	# of samples	10

XVI Calibrator

c a l i b r a t o r

Rat Skeletal Troponin I (sTnl) was purified from rat skeletal muscle. This analyte was calibrated against internal controls, diluted, and pooled to a final concentration of 4 µg/mL for sTnl.

XVII References

references

1. Baldwin AS Jr, Kittler EL, Emerson CP Jr. Structure, evolution, and regulation of a fast skeletal muscle troponin I gene. *Proc Natl Acad Sci U S A*. 1985 Dec;82(23):8080-4.
2. Brotto MA, Biesiadecki BJ, Brotto LS, Nosek TM, Jin JP. Coupled expression of troponin T and troponin I isoforms in single skeletal muscle fibers correlates with contractility. *Am J Physiol Cell Physiol*. 2006 Feb;290(2):C567-76.
3. Simpson JA, Van Eyk J, Iscoe S. Respiratory muscle injury, fatigue and serum skeletal troponin I in rat. *J Physiol*. 2004 Feb 1;554(Pt 3):891-903.
4. Simpson JA, Labugger R, Hesketh GG, D'Arsigny C, O'Donnell D, Matsumoto N, Collier CP, Iscoe S, Van Eyk JE. Differential detection of skeletal troponin I isoforms in serum of a patient with rhabdomyolysis: markers of muscle injury? *Clin Chem*. 2002 Jul;48(7):1112-4.
5. Shi Q, Ling M, Zhang X, Zhang M, Kadujevic L, Liu S, Laurino JP. Degradation of cardiac troponin I in serum complicates comparisons of cardiac troponin I assays. *Clin Chem*. 1999 Jul;45(7):1018-25.
6. Filatov VL, Katrukha AG, Bulargina TV, Gusev NB. Troponin: structure, properties, and mechanism of functioning. *Biochemistry*. 1999 Sep;64(9):969-85.
7. Zot AS, Potter JD. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. *Annu Rev Biophys Biophys Chem*. 1987;16:535-59.

Summary Protocol

MSD 96-well MULTI-ARRAY Rat Skeletal Troponin I Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Rat Skeletal Troponin I Assay.

Step 1 : Sample and Reagent Preparation

Bring appropriate diluents and plates to room temperature.

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

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

Prepare an 8-point standard curve using supplied calibrators:

- The Calibrator should be diluted in Diluent 7 + Additives.
- Dilute the stock Calibrator 1:20 in Diluent 7 + Additives then perform a series of 3-fold dilution steps and a no calibrator blank.
- Let calibrator and samples in Diluent 7 + Additives incubate 30 min before addition to plate.

Prepare Detection Antibody Solution by diluting the 50X Detection Antibody to 1X 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 surfactant) with deionized water.

Step 2 : Add Assay Diluent GF1

Dispense 25 μ L/well Diluent 7 + additives.

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

Step 3 : Add Sample or Calibrator

Dispense 25 μ L/well Calibrator or Sample.

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

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

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

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

Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

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

Analyze plate on SECTOR instrument.

