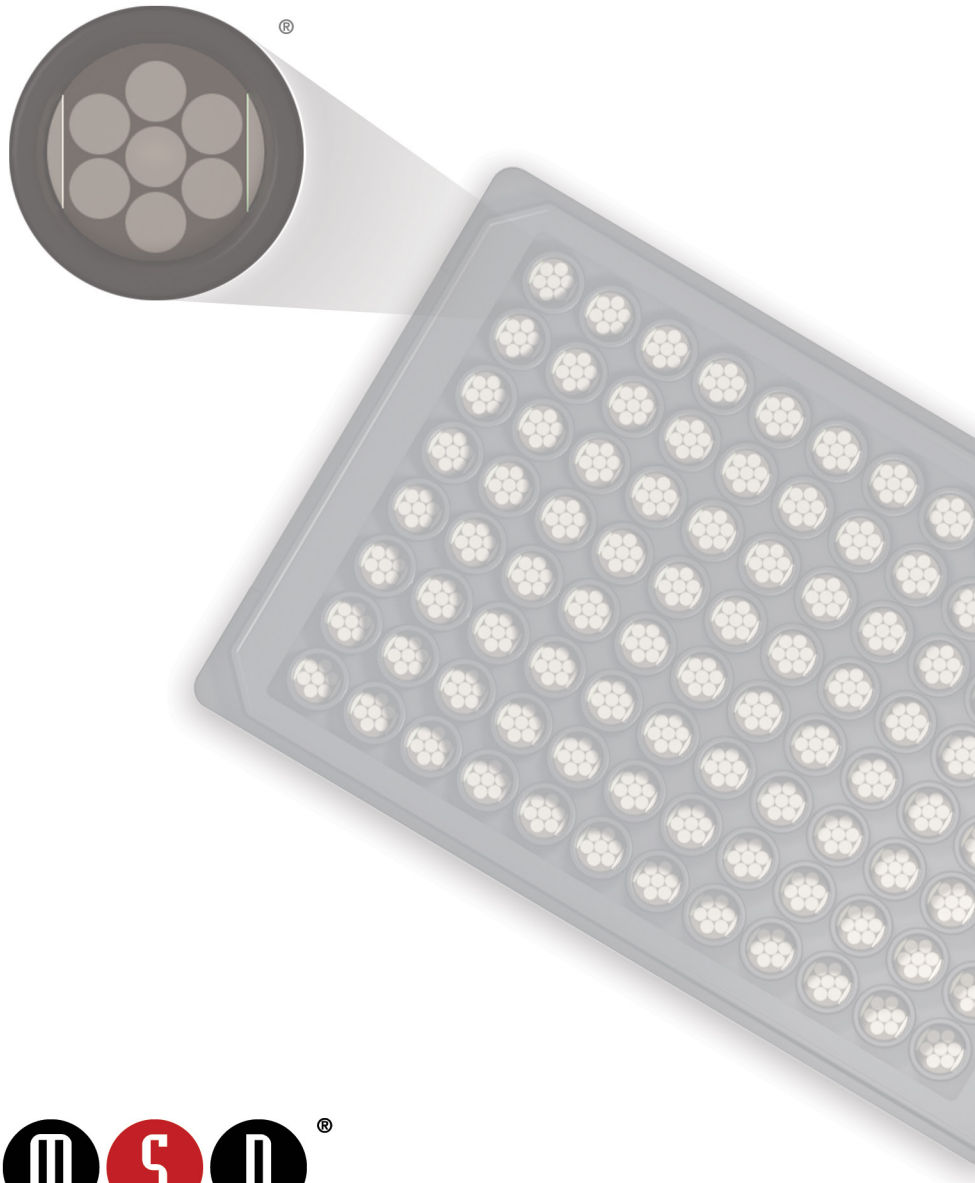
MSD® MULTI-SPOT Assay System

Rat Skeletal Troponin I (fast-twitch) Kit

T-PLEX®



	Catalog No
Individual Assay Kit	K153IMC

MSD®

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MSD Toxicology Assays

Rat Skeletal Troponin I (fast-twitch) Kit

For use with rat 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.[2] Troponins are excellent biomarkers for myocardial injury in cardiotoxicity because of their demonstrated tissue specificity.[3]

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

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Rat Skeletal Troponin I (fast-twitch) assay is a sandwich immunoassay (Figure 1). MSD provides a plate precoated with capture antibodies on independent and well-defined spots, as shown in the layout below. 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 kit has been qualified according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[4]

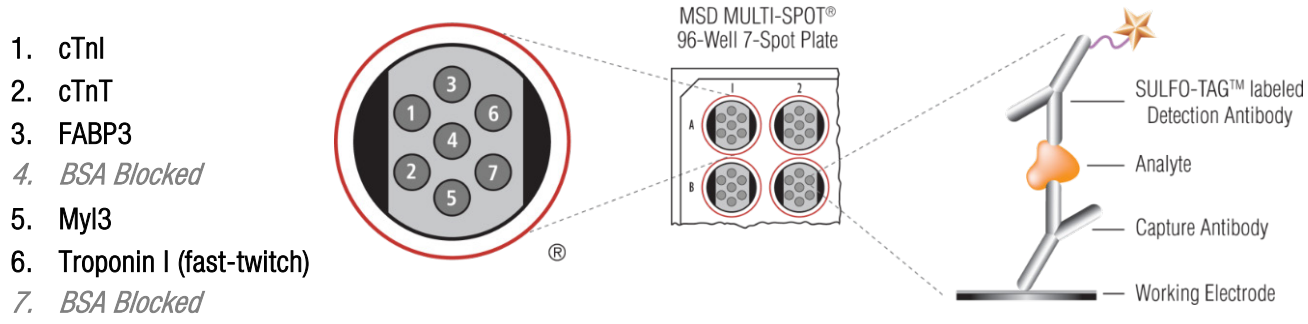

Figure 1. A multiplex plate spot diagram showing the placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Kit Components

Reagents Supplied

Kit reagents are listed in Table 1.

Table 1. Rat Skeletal Troponin I (fast-twitch) Kit reagents that are supplied with the Kit

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Muscle Injury Panel 1 (rat) Plate	2–8 °C	N75181A-1	7-spot	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant.
Rat Skeletal Troponin I (fast-twitch) Calibrator (20X)	≤–70 °C	—	1 vial (20 µL)	1 vial	5 vials	25 vials	Recombinant rat protein in diluent, buffered and frozen. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
Anti-rat Troponin I (fast-twitch) Antibody (50X)	2–8 °C	D23IM-2	75 µL	1 vial	—	—	SULFO-TAG conjugated detection antibody
		D23IM-3	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 7	≤–10 °C	R54BB-4	5 mL	2 bottles	—	—	Diluent for samples and Calibrators
		R54BB-3	50 mL	—	1 bottle	5 bottles	
DTT (25 mM)	≤–10 °C	—	1 mL	1 vial	1 vial	5 vials	Diluent 7 additives
EDTA pH 8.0 (0.5M)	RT	—	4 mL	1 bottle	1 bottle	5 bottles	Diluent 7 additives
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	Buffer to catalyze the electrochemiluminescent 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 them as directed immediately.
- 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 500–1,000 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 7 into suitably sized aliquots before refreezing. Some components in Diluent 7 are not soluble below room temperature. Diluents can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay

Prepare Diluent 7 plus Additives

For the Rat Skeletal Troponin I (fast-twitch) assay, samples, and calibrators must be diluted in Diluent 7 to which EDTA and DTT have been added. EDTA and DTT additive stocks are provided at the concentrations shown in Table 2

Table 2: EDTA and DTT additive stocks

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 calibrator for the Rat Skeletal Troponin I (fast-twitch) Kit at a 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve 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. To view the actual concentration of the assay, 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 K153IMC 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 12 µL of stock calibrator to 228 µL of Diluent 7 plus additives. Mix well by vortexing.
2) Prepare the next calibrator by transferring 80 µL of the highest standard to 160 µL of Diluent 7 plus additives. Mix well by vortexing. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 7 plus additives as the zero calibrator.

Dilute Samples

For normal rat serum and plasma samples, no dilution is necessary. MSD recommends a 2- to 20-fold dilution for samples from rats that have suffered a muscle injury. For example, when running 4-fold diluted samples in duplicate, add 25 µL of sample to 75

µL of Diluent 7 plus additives. We recommend running at least two replicates per sample. The kit includes diluent sufficient for running samples in duplicates. 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:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat Skeletal Troponin I (fast-twitch) Antibody
- ❑ 2,940 µL of Diluent 30

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 7 plus Additives

- ❑ Add 25 µL of Diluent 7 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.

Analysis of Results

The calibrators should be run minimally 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 to 4 logs) which allows accurate quantification 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.

Assay Components

Calibrators

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

Antibodies

The antibody source species are described in Table 3.

Table 3. Antibody source species

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
Troponin I (fast-twitch)	Mouse Monoclonal	Mouse Monoclonal

References

1) Gomes AV, et al. The role of Troponin in muscle contraction. IUBMB Life. 2002 Dec;54(6):323-33.
2) Marston SB, Redwood CS. Modulation of thin filament activation by breakdown or isoform switching of thin filament Proteins. Circ. Res. 2003 Dec 12;93(12):1170-8.
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) Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28.

Summary Protocol

Rat Skeletal Troponin I (fast-twitch) Kit

MSD provides this summary protocol for your convenience.

Please read the entire detailed protocol before performing the Rat Skeletal Troponin I Kit.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Diluent 7 plus additives by diluting the provided DTT (100X) and EDTA (16.7X) stock solutions to 1X concentration in Diluent 7.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator blend 20-fold in Diluent 7 plus additives.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ If necessary, dilute samples in Diluent 7 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 7 plus Additives

- ❑ Add 25 µL of Diluent 7 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

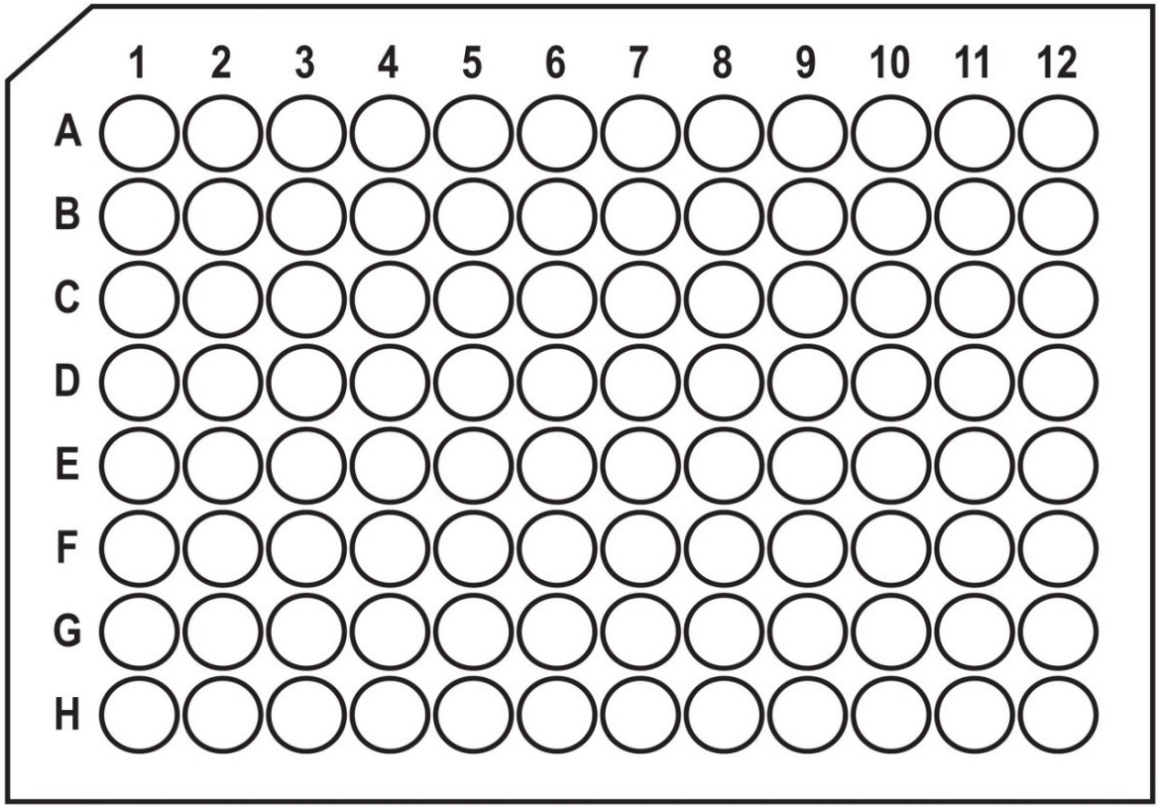

Figure 2. Plate diagram; a similar plate layout can be created in Excel and easily imported into DISCOVERY WORKBENCH software.