MSD® 96-Well MULTI-ARRAY®

Rat Troponin ITC Complex Assay

Summary

Rat Troponin ITC Complex assay measures one of the common biomarkers of cardiac injury. This product insert describes a recommended protocol for running the assay in singleplex format.
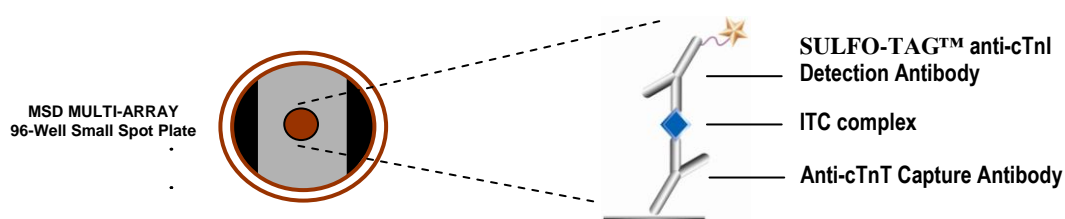
Figure 1. Capture Antibody is pre-coated on a small Spot MSD MULTI-ARRAY plate. Calibrator solutions or sample are incubated in the MULTI-ARRAY plate. The analyte binds to the Capture Antibody spot and is detected using a specific Detection Antibody labeled with MSD SULFO-TAG™ reagent.

Reagents Provided

Storage

Detection Antibody	Antibody labeled with MSD SULFO-TAG reagent. The detection antibody is provided as a 50X concentrated stock solution.	2-8 $^{\circ}$C
Calibrator	Rat Troponin ITC Complex Calibrator is supplied at 5 µg/mL.	≤ -70 $^{\circ}$C
Diluent 7	Protein and animal derived material medium for Assay Diluent and dilution of Calibrators and samples	≤ -10 $^{\circ}$C
Diluent 5	Contains blocking and stabilizing agents.	≤ -10 $^{\circ}$C
Read Buffer T	4X Read Buffer T with surfactant	RT
MULTI-ARRAY Plate	96-well MSD plate coated with capture antibody	2-8 $^{\circ}$C

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 (PBST) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of accurately dispensing 10 to 150 μL into a 96-well microtiter plate
- Automated plate washer, or other efficient multi-channel pipetting equipment for washing 96-well plates
- Adhesive plate seals
- Microtiter plate shaker

Reagent Preparation

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

Prepare Calibrator Solutions:

Calibrator for the Rat Troponin ITC complex assay is supplied at 20-fold higher concentration than the recommended highest Calibrator. An 8-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator.

One 15 μL vial is supplied per plate at 5000 ng/mL.

To prepare an 8-point calibration curve for up to 5 replicates:
1) Prepare the highest Calibrator by adding 10 μL of the Calibrator stock vial to 190 μLof Diluent 7.
2) Prepare the next Calibrator by transferring 50 μL of the diluted Calibrator to 150 μL of Diluent 7. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th dilution is Diluent 7 alone (e.g. zero Calibrator).

This Yields the following Calibrator concentrations:

Calibrator (ng/mL)	ITC (ng/mL)
Calibrator 7	250
Calibrator 6	62.5
Calibrator 5	15.63
Calibrator 4	3.91
Calibrator 3	0.98
Calibrator 2	0.24
Calibrator 1	0.06
Zero Calibrator	0

Calibrator solutions should be prepared no more than 20 minutes before use.

Dilution of samples (optional):

Some rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. If this is the case, Diluent 7 should be used to dilute samples. For serum and plasma from normal rats, no dilution is necessary. However, for rats that have suffered a cardiac injury, 2-20X dilution of serum or plasma samples is sometimes necessary. For cardiac tissue lysates or homogenates, 50-500X dilution may be required.

Prepare Detection Antibody Solution:

The Detection Antibody is provided as individual stock solution at 50X concentration. The working Detection Antibody Solution should contain 1X concentration of each Detection Antibody. For each plate used, dilute 60 μL of each stock Detection Antibody into a final volume of 3 mL of Diluent 5. Detection Antibody Solution should be kept in the dark as some antibodies may be light sensitive.

***Prepare Read Buffer*:**
The Read Buffer should be diluted 1:4 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.

Protocol

1. ***Addition of Diluent 7:*** Dispense 25 µL of Diluent 7 into each well. Pipette to the bottom of the plate so as to allow the fluid to cover the entire bottom of the well. A slight tap may be necessary to allow the fluid to settle to the bottom. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (300-1000 rpm) at room temperature.

2. ***Addition of Sample or Calibrator:*** Dispense 25 µL of Calibrator or Sample Solution into a separate well 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 Detection Antibody Solution:*** Wash the plate 3X with PBS + 0.05% Tween-20. Dispense 25 µL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

4. ***Wash and Read:*** Wash the plate 3X with PBS + 0.05% Tween-20. Add 150 µL 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 addition of Read Buffer. Note: Bubbles in the fluid will interfere with reliable reading of the MULTI-ARRAY plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

Additional Notes

1. ***Sample Matrices:*** Because the MULTI-ARRAY plate and kit are custom products, no particular claims are made regarding the compatibility with specific sample matrices. In general, these plates have been found to work well to measure analyte levels in a wide range of samples, including serum and plasma samples. Sample dilution and/or spike recovery studies in the sample matrix of interest should be carried out to verify acceptable performance in the matrix.

2. ***Assay specificity:*** The ITC singleplex assay detects only the ITC Complex, and does not cross-react with either cTnI or cTnT monomers.

3. ***ITC concentration:*** The concentration of the ITC Calibrator is expressed relative to the cTnI portion of the Complex only. The stock Calibrator at 5000 ng/mL contains 5000 ng/mL of cTnI, and this corresponds to approximately 11000 ng/mL of ITC.

Topics of Interest

1. ***Background signal and negative signals:*** The output signal produced by electrochemiluminescence assays is in units of counts of light measured by a charge-coupled device (CCD) camera or photodiode. As with any measurement technique, there is a certain amount of normal variation in this signal (instrument noise) which sets the threshold for the lowest levels of signal that can be measured (noise floor). This variation is different depending upon the size of the working electrode with typical values of about 10 counts for 96-well small spot and 96-well 4-spot plates, 15 counts for 96-well 7-spot plates, and 30 counts for 96-well 10-spot plates. When the background signal of an assay approaches the noise floor (i.e. the mean signal of negative controls or sample blanks is close to zero), it is possible to observe negative counts for some wells.

2. ***Signal Levels:*** The camera system is linear over nearly a 6 log-dynamic range. The highest achievable signals on the SECTOR Imager 6000 and 2400 are between 1.0 and 2.0 million counts. If the signals from the highest point on the calibration curve are not approaching 1.0 million counts, the high end of the calibration curve may be extended. The lowest observed signals using Read Buffer T (2X) are between 10 and 50. Negative signal values may occur due to instrument noise, omission or usage of the incorrect Read Buffer, or incorrect amount of Detection Antibody.

3. ***Fitting methods:*** To utilize the quantitative value of electrochemiluminescent detection, a titration curve is produced using a known standard. 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. MSD's assays have a wide dynamic range (typically 3-5 logs) which allows accurate quantitation in many samples without the need to dilute prior to running the assay. MSD recommends using software to fit the data that 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. An alternative analysis approach is to subtract the background signal from all data points, and then use a linear model to fit the data. The disadvantage of this approach is that a skewed calibration curve may be created if the background signal used for subtraction is not an appropriate indicator of background signal over the complete curve. Also, negative numbers may be produced if background-corrected signal values are less than the instrument noise observed in signals at the low end of the curve.

4. ***Reverse pipetting:*** Most manual hand pipettes have two plunger positions for pipetting liquids. The first position is calibrated to allow aspiration and dispensing of user-specified amounts of liquid and the second (blow-out) position enables the user to expel any residual liquid after the pipette has been pushed to the first position. When a pipette is used to dispense liquid by moving the plunger to the first position followed by the second (blow-out) position, bubbles may be created in the dispensed liquid. The reverse pipetting technique is designed to allow precise pipetting while avoiding the creation of bubbles. The technique is to push the pipette plunger past the first position to the second position prior to aspirating liquid into the tip, thereby aspirating slightly more liquid than the desired volume (overdraw). In order to dispense the liquid from the tip, the pipette plunger is pushed to the first position only. This allows precise dispensing without the introduction of bubbles. When using the reverse pipetting technique, it is important not to overdraw excess liquid into the pipette mechanism.

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MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the assay.

STEP 1: **Sample and Reagent Preparation**
Bring appropriate diluents and plates to room temperature.
If necessary, samples should be diluted in Diluent 7.
Prepare an 8-point calibration curve using the supplied Calibrator:
- The Calibrator should be diluted in Diluent 7
- Calibrator is supplied at 20-fold higher concentration than the recommended highest Calibrator concentration. Dilute the Calibrator by 20-fold in Diluent 7, then perform a series of 4-fold serial dilution steps.

Prepare a working Detection Antibody Solution by diluting the Detection Antibody to 1X concentration in 3.0 mL of Diluent 5 (per plate). Detection Antibody is supplied at 50X concentration.
Prepare 20 mL of 1X Read Buffer T by diluting 4X MSD Read Buffer T 1:4 with deionized water.

STEP 2: **Add Diluent 7**
Dispense 25 μL/well Diluent 7.
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

STEP 3: **Add Sample or Calibrator**
Dispense 25 μL/well Calibrator or Sample.
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

STEP 4: **Wash and Add Detection Antibody**
Wash plate 3X with PBS-0.05% Tween-20.
Dispense 25 μL/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-0.05% Tween-20.
Dispense 150 μL/well 1X Read Buffer T.
Analyze plate on SECTOR instrument.