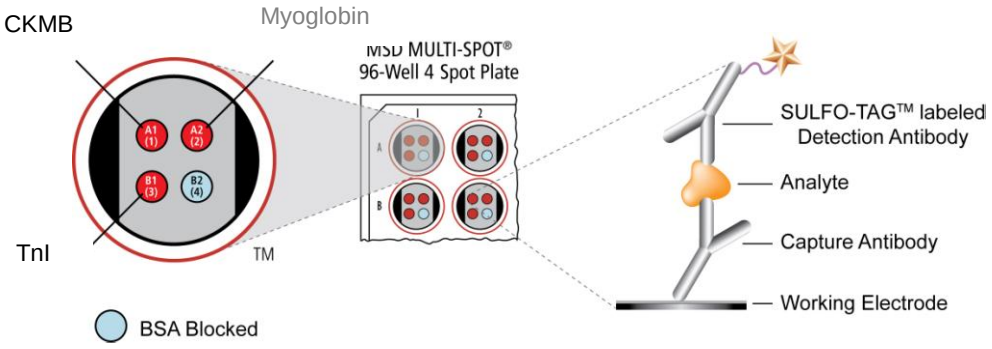
# *MSD 96-Well MULTI-SPOT Human Cardiac I Assay*

*This protocol has been optimized for detection of CKMB, and troponin-I in human serum samples.*

## *MSD® Materials*

| | Storage |
|---|---|
| ❑ Read Buffer T (4X) | RT |
| ❑ MULTI-SPOT® 96-well 4 Spot Human Cardiac Panel I Plate | 2-8 °C |
| ❑ SULFO-TAG™ Anti-CKMB Antibody (50X)[1] | 2-8 °C |
| ❑ SULFO-TAG Anti-Troponin-I Antibody (50X)[1] | 2-8 °C |
| ❑ Diluent 24 | ≤-10 °C |
| ❑ Diluent 25[2] | ≤-10 °C |
| ❑ Cardiac-I High Calibrator[3] | ≤-70 °C |




**The SECTOR® Imager data file will identify spots according to their well location, not by the coated capture antibody name.**

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

[2] The Diluent 25 contains pooled serum which has been tested and found to be negative for HIV-1 and HIV-2 antibodies and HCV antibody and nonreactive for HBsAg, HIV-1 RNA, HCV RNA, and STS. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

[3] The Calibrator blend in this kit is derived from human source material which has been tested and found to be negative for HBsAg, HIV-1 and HIV-2 antibodies, HCV and syphilis. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

## Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Read Buffer
- ❑ Phosphate buffered saline for plate washing
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Liquid handling equipment that must accurately dispense 25 and 150 µL into a 96-well micro plate

**Notes:**

## Protocol at a Glance

**The protocol can be completed in approximately 3.5 hours**.

1. Add Diluent 24 to each well.
2. Add calibrator or sample and incubate for 2 hours with shaking.
3. Wash
4. Add Detection Antibody Reagent to each well and incubate for 1 hour with shaking.
5. Wash.
4. Add Read Buffer and Read plate.

## Preparation Instructions

**Prepare Calibrators:**

1. MSD recommends the preparation of an 8-point calibration curve consisting of at least 2 replicates of each point. Each well requires 25 µL of calibrator. Thaw the Diluent 25 and Cardiac-I High Calibrator (STD-01). Prepare serial dilutions of the Cardiac-I High Calibrator in Diluent 25. For the assay, an 8-point standard curve is recommended with 5-fold serial dilution steps and Diluent 25 alone for the 8$^{th}$ point.

   Concentrations of the 8-point standard curve:

| Standard | CKMB (ng/mL) | TnI (ng/mL) | Dilution Factor |
|---|---|---|---|
| **STD-01** | 550 | 33 | |
| **STD-02** | 110 | 6.6 | 5 |
| **STD-03** | 22 | 1.3 | 5 |
| **STD-04** | 4.4 | 0.26 | 5 |
| **STD-05** | 0.88 | 0.053 | 5 |
| **STD-06** | 0.18 | 0.011 | 5 |
| **STD-07** | 0.035 | 0.0021 | 5 |
| **STD-08** | 0 | 0 | n/a |

*Calibrators should be kept at 4°C (for up to 4 hours) if not used immediately.*

2. To prepare the 8-point standard curve:

a) Cardiac-I High Calibrator is utilized as the highest Calibrator point.

b) Prepare the next Calibrator by transferring 40 µL of the Cardiac-I High Calibrator to 160 µL of Diluent 25.

c) Prepare the next Calibrator by transferring 40 µL of the diluted Calibrator to 160 µL of Diluent 25. Repeat 5-fold serial dilutions 4 additional times to generate 7 Calibrators.

d) Reserve 200 µL of Diluent 25 to be used for STD-08.

**Notes:**

**Prepare Detection Antibody Reagent:**

1. Each well will require 25 µL of Detection Antibody Reagent. Prepare 3 mL per plate. Diluent 24 can be refrozen up to 3 times.
2. In a 15 mL tube combine:
    a. 2.88 mL Diluent 24
    b. 60 µL of 50X SULFO-TAG Anti-CKMB Antibody (final concentration: 1X)
    c. 60 µL of 50X SULFO-TAG Anti-TnI Antibody (final concentration: 1X)
3. Detection Antibody Reagent is stable at room temperature for a few hours.

**Dilute Read Buffer:**

1. Determine total number of wells in the experiment. Each well will receive 150 µL of Read Buffer T. Prepare an extra 20%.
2. Dilute 4X Read Buffer T to 1X with deionized water.
3. Diluted Read Buffer may be stored at room temperature for later use.

## *Assay Protocol*

Begin with a MULTI-SPOT 96-well 4-Spot Human Cardiac I Plate.

1. Add 25 µL/well of Diluent 24.
2. Add 25 µL/well calibrator or sample and incubate at room temperature with shaking for 2 hours.
3. Wash plates 3 times with PBS.
4. Add 25 µL/well of Detection Antibody Reagent (solution of Diluent 24 containing two diluted SULFO-TAG antibodies) and incubate at room temperature with shaking for 1 hour.
5. Prepare SECTOR instrument such that the plate can be read immediately following Read Buffer addition.
6. Wash plates 3 times with PBS.
7. Add 150 µL/well 1X Read Buffer T. Avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting is recommended.

*Bubbles introduced to the well during Read Buffer addition will interfere with reliable imaging of the plate.*