

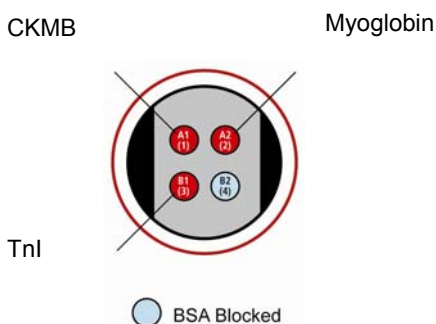
MSD[®] 96-Well MULTI-SPOT[®] Human Cardiac I Assay

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

Storage

MSD Materials

| | |
|--|---------|
| □ 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-Myoglobin 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.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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

Protocol at a Glance

The protocol can be completed in approximately 2.5 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 8th point.

Concentrations of the 8-point standard curve:

| Standard | Myoglobin (ng/mL) | CKMB (ng/mL) | TnI (ng/mL) | Dilution Factor |
|---------------|-------------------|--------------|-------------|-----------------|
| STD-01 | 10000 | 550 | 33 | |
| STD-02 | 2000 | 110 | 6.6 | 5 |
| STD-03 | 426 | 22 | 1.3 | 5 |
| STD-04 | 80 | 4.4 | 0.26 | 5 |
| STD-05 | 16 | 0.88 | 0.053 | 5 |
| STD-06 | 3.2 | 0.18 | 0.011 | 5 |
| STD-07 | 0.64 | 0.035 | 0.0021 | 5 |
| STD-08 | 0 | 0 | 0 | n/a |

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.

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



Notes:

Prepare Detection Antibody Reagent:

1. Each well will require 25 μ L of Detection Antibody Reagent. Prepare 3 mL per plate. Assay Diluent can be refrozen up to 3 times.
2. In a 15 mL tube combine:
 - a. 2.82 mL Diluent 24
 - b. 60 μ L of 50X SULFO-TAG Anti-CKMB Antibody (final concentration: 1X)
 - c. 60 μ L of 50X SULFO-TAG Anti-MYO Antibody (final concentration: 1X)
 - d. 60 μ L 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 μ L 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 μ L/well of Diluent 24.
2. Add 25 μ L/well calibrator or sample and incubate at room temperature with shaking for 2 hours.
3. Wash plates 3 times with PBS.
4. Add 25 μ L/well of Detection Antibody Reagent (solution of Diluent 24 containing three 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 μ L/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.

