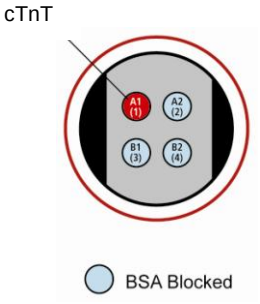
MSD® 96-Well MULTI-ARRAY®
Human Cardiac Troponin T Assay

The following assay protocol has been optimized for the quantitative measurement of cardiac troponin T (cTnT) in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X)	RT
❑ MULTI-SPOT® 96-well 4 Spot Human cTnT plate	2-8 °C
❑ SULFO-TAG™ Anti-hcTnT Antibody (50X)[1]	2-8 °C
❑ Diluent 22	≤-10 °C
❑ Diluent 7	≤-10 °C
❑ Human Cardiac Troponin T Calibrator[2] (25 ng/mL)	≤-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 Calibrator 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.

Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Read Buffer
- ❑ Phosphate buffered saline + 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Liquid handling equipment, or other efficient multi-channel pipetting equipment that must accurately dispense 25 and 150 µL into a 96-well micro plate
- ❑ Automatic plate washer or multi-channel pipette for washing 96-well plates

Protocol at a Glance

Read the entire detailed instructions before beginning work.

The protocol can be completed in approximately 1.5 hours.

Step 1. Add 25 µL of Detection Antibody Solution.
Add 25 µL of Samples or Calibrator, incubate 1 hour, wash.

Step 2. Add 150 µL of Read Buffer, read plate and analyze data.

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 will require 25 µL of Calibrator. Thaw the Diluent 7 and the Calibrator (25 ng/mL) and prepare the desired concentrations by making serial dilutions using the Calibrator Stock and Diluent 7.

   A recommended Calibrator dilution procedure is listed below:
   This will prepare sufficient reagent for 3 replicates of each Calibrator concentration.
   - *Prepare 6 serial dilutions starting with the supplied Calibrator stock (25 ng/mL) and diluting by a factor of 4; add 40 µL of the higher Calibrator concentration to 120 µL of Diluent 7. Vortex thoroughly.*
   - *This will create seven Calibrators with 25, 6.25, 1.56, 0.39, 0.1, 0.02, 0.006 ng/mL of Troponin T.*
   - *Reserve 100 µL of Diluent 7 as the 8th (zero) Calibrator.*

2. Calibrators should be stored at 4 ^{o}C (for up to 4 hours) if not used immediately. Avoid repeated freeze-thawing of 25 ng/mL Calibrator stock. Aliquot into single-use volumes and store at ≤-70 ^{o}C. Diluent 7 may be refrozen twice.

Notes:

Prepare the 1X Detection Antibody Solution:

1. In a 15 mL tube combine:
   - ❑ 60 µL of 50X SULFO-TAG Anti-hcTnT Antibody
   - ❑ 2.94 mL of Diluent 22
2. This will yield 3 mL of diluted Detection Antibody Solution at the working concentration with sufficient volume for one plate.

Diluent 22 can be refrozen up to 3 times.

Dilute Read Buffer:

In a 50 mL tube combine (per plate):
- ☐ 5 mL 4X Read Buffer T
- ☐ 15 mL deionized water

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human cTnT plate.

1. Add 25 µL/well of Detection Antibody Solution.
2. Add 25 µL/well Calibrator or sample, cover with an adhesive plate seal, and incubate at room temperature with shaking for 1 hour.
3. Prepare SECTOR Imager so that the plate can be read immediately after Read Buffer addition.
4. Wash plates 3 times with PBS-T.
5. Add 150 µL/well 1X Read Buffer T. Avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting is recommended.
6. Analyze immediately with SECTOR Imager.

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