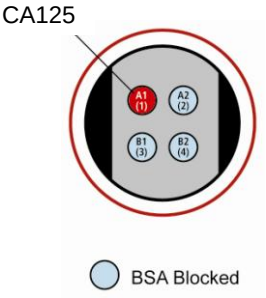
MSD® 96-Well MULTI-ARRAY® Human Cancer Antigen 125 Assay

The following assay protocol has been optimized for analysis of human cancer antigen 125 (CA125) in human serum or plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 4 Spot Human CA125 Plate(s)	2-8°C
❑ SULFO-TAG™ Anti-hCA125 Antibody (50X)	2-8°C
❑ Diluent 7	≤-10°C
❑ Diluent 8	≤-10°C
❑ Diluent 9	≤-10°C
❑ Human Cancer Antigen 125 Calibrator[1] (50 kU/mL)	≤-70°C



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

[1] The Calibrator in this kit is derived from human source material which has been tested and found to be negative for HIV-1, HIV-2, HCV antibodies and HBsAg. 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 + 0.05% Tween-20 (PBST) for plate washing
- Adhesive plate seals
- Microtiter plate shaker
- Plate washer, or other efficient multi-channel pipetting equipment for washing 96-well plates
- Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 and 150 µL into a 96-well microplate

Notes:

Read the entire detailed instructions before beginning work.

The assay protocol was optimized for human serum samples. For significantly different sample matrices, it is recommended to use a Calibrator diluent that is similar to the sample matrix (e.g. lysis buffer + carrier protein).

Protocol at a Glance

The protocol can be completed in approximately 5.5 hours if each reagent is prepared during the preceding incubation. This time can reduced to **4.5 hours if the blocking reagent is added the night before**.

Step 1. Add Blocking solution, incubate 1-2 hours, wash.

Step 2. Add 25 µL of Diluent 7.
Add 25 µL of samples or Calibrator, incubate 2 hours, wash.

Step 3. Add 25 µL of Detection Antibody, incubate 2 hours, wash.

Step 4. Add 150 µL of Read Buffer and analyze plate.

Preparation Instructions

Solutions containing Blocker A should be kept at 4ºC and discarded after 14 days.

Prepare Blocker A Solution:

Follow instructions included with the Blocker A Kit.

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be run. Each well will require 25 µL of Calibrator. Thaw one vial of human Cancer Antigen 125 Calibrator stock solution and prepare the required Calibrator dilution series using the stock solution and Diluent 9.

Notes:

- A recommended Calibrator dilution procedure is listed below for up to 4 replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
- *Prepare 200 µL of a high Calibrator containing 5000 U/mL CA125 by combining 20 µL of CA125 stock solution at 50 kU/mL with 180 µL of Diluent 9.*
- *Prepare 6 additional 1:4 serial dilutions, beginning with the high Calibrator, by adding 50 µL of the Calibrator to 150 µL Diluent 9.*
- *This will create 7 Calibrators with 5000, 1250, 313, 78, 20, 4.9, and 1.2 U/mL of CA125.*
- *The recommended 8th dilution is Diluent 9 alone (e.g. zero Calibrator).*

❖ Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately.

2. Calibrators are stable at room temperature for a few hours.

Prepare Detection Antibody Reagent:

1. Each well requires 25 µL of Detection Antibody Reagent. Prepare 3 mL per plate.
2. In a 15 mL tube combine:
   a. 2.94 mL Diluent 8
   b. 60 µL of 50X SULFO-TAG Anti-hCA125 Antibody (final concentration: 1X)

Dilute Read Buffer:

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

In a 50 mL tube, combine (per plate):

- ☐ 5 mL 4X Read Buffer T
- ☐ 15 mL deionized water

Assay Protocol

Begin with a MULTI-SPOT 96-well 4l Spot Human CA125 plate.
No pre-treatment is necessary.

1. Add 150 µL/well of Blocker A Solution and incubate at room temperature for 1-2 hours.

2. Wash plates 3 times with phosphate buffered saline + 0.05% Tween-20 (PBST).

3. Add 25 µL/well of Diluent 7.
   Add 25 µL/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBST.

5. Add 25 µL/well Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.

6. Wash plates 3 times with PBST.

7. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.

8. Add 150 µL/well 1X Read Buffer T.

9. Analyze immediately with SECTOR Imager.

Notes:

Avoid bubbles while adding the Read Buffer; it will interfere with accurate reading of the plate.