

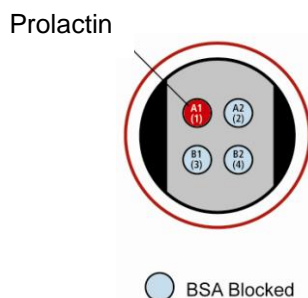
MSD[®] 96-Well MULTI-ARRAY[®] Human Prolactin Assay

The following assay protocol has been optimized for analysis of prolactin in human serum or plasma samples.

Storage

MSD Materials

<input type="checkbox"/> Read Buffer T (4X), with surfactant	RT
<input type="checkbox"/> Blocker A Kit	RT
<input type="checkbox"/> MULTI-SPOT [®] 96-well 4 Spot Prolactin Plate(s)	2-8 °C
<input type="checkbox"/> SULFO-TAG [™] Anti-Prolactin Antibody (50X)	2-8 °C
<input type="checkbox"/> Diluent 100	2-8 °C
<input type="checkbox"/> Diluent 7	≤-10 °C
<input type="checkbox"/> Diluent 11	≤-10 °C
<input type="checkbox"/> Human Prolactin Calibrator (1µg/mL)	≤-70 °C



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

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



Notes:

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
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 and 150 μL into a 96-well microplate

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 be 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.

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).

Preparation Instructions

Prepare Blocker A solution:

Follow instructions included with the Blocker A Kit.

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

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 Prolactin Calibrator stock solution and prepare the required Calibrator dilution series using the stock solution and Calibrator Diluent.



Notes:

- A recommended Calibrator dilution procedure is listed below for up to 3 replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
 - Prepare 200 μL of a high Calibrator containing 100 ng/mL Prolactin by adding 20 μL of the Calibrator stock solution at 1 $\mu\text{g}/\text{mL}$ to 180 μL Diluent 100.
 - Prepare 6 additional 1:5 serial dilutions, beginning with the high Calibrator, by adding 50 μL of the Calibrator to 200 μL Diluent 100.
 - This will create 7 Calibrators with 100, 20, 4, 0.8, 0.16, 0.032 and 0.006 ng/mL of Prolactin
 - The recommended 8th dilution is Diluent 100 alone (i.e. 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 at least a few hours.

Prepare Samples:

**Dilute serum or plasma samples 10-fold in Diluent 100.
Each well will require 25 μL of diluted sample.**

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 11
 - b. 60 μL of 50X SULFO-TAG Anti-Prolactin Antibody (final concentration: 1X)

Prepare Read Buffer Solution:

- 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.



Notes:

Assay Protocol

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

1. Add 150 μ L/well of Blocker A and incubate at room temperature for 1-2 hours.
2. Wash plates 3 times with phosphate buffered saline (PBS)
3. Add 25 μ L/well of Diluent 7.
Add 25 μ L/well Calibrator or 10-fold diluted sample and incubate at room temperature with shaking for 2 hours.
4. Wash plates 3 times with PBS.
5. Add 25 μ L/well Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.
6. Wash plates 3 times with PBS.
7. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.
8. Add 150 μ L/well 1X Read Buffer T. Avoid bubbles.
9. Analyze immediately with SECTOR Imager.

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

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