

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

The following assay protocol has been optimized for the quantitative measurement of Progesterone in human serum.

Storage

MSD Materials

<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> MULTI-SPOT [®] 96-well Human Progesterone Plate ¹	2-8 °C
<input type="checkbox"/> SULFO-TAG [™] Progesterone (1 µg/mL)	2-8 °C
<input type="checkbox"/> Diluent 22	≤-10 °C
<input type="checkbox"/> Human Progesterone Calibrator (Set of 8 vials ²)	-20 °C

Other Materials & Equipment (not supplied)

- Deionized water for diluting Read Buffer
- Phosphate Buffered Saline for plate washing
- Adhesive plate seals
- Microtiter plate shaker
- Automated 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 micro plate

¹ The Kit may be supplied with Multi –spot 96 well 4 spot Human Fertility panel plate

² Each vial contains enough material for **two** plates.

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



Protocol at a Glance

Notes:

The protocol can be completed in approximately 1.5 hours.

Read the entire detailed instructions before beginning work.

1. Add Detection Reagent to each well.
2. Add Calibrator or sample and incubate for 1 hour with shaking.
3. Wash.
4. Add Read Buffer and Read plate.

Preparation Instructions

Prepare Calibrators:

1. Thaw one set of Calibrators: one vial contains a sufficient volume for two plates containing duplicate samples (25 μ L/well, 100 μ L provided). Thawed Calibrator can be kept at room temperature for a few hours, and can be refrozen up to 3 times.
2. At least two replicates of the Calibrator should be included on each plate to produce a well-defined calibration curve.

Prepare Detection Reagent:

1. Determine total number of wells in the experiment. Each well will require 25 μ L of Detection Reagent. Thaw an appropriate amount of Diluent 22 for the experiment. Diluent 22 can be refrozen up to 3 times.
2. Prepare Detection Reagent as follows:
 - Dilute labeled Progesterone (stock – 1 μ g/mL) to a final concentration of 2 ng/mL
3. Detection 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

Notes:

Begin with a MULTI-SPOT 96-well Human Progesterone Plate.

1. Add 25 μ L/well of Detection Reagent (solution of Diluent 22 containing diluted SULFO-TAG progesterone).
2. Add 25 μ L/well Calibrator or sample and incubate at room temperature with shaking for 1 hour.
3. Prepare SECTOR[®] instrument such that the plate can be read immediately following Read Buffer addition.
4. Wash plates 3 times with PBS.
5. Add 150 μ L/well 1X Read Buffer T. Avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting is recommended.

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

