

# MSD<sup>®</sup> 96-Well MULTI-ARRAY<sup>®</sup> 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 <sup>®</sup> 96-well Human Progesterone Plate <sup>1</sup>	2-8 °C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Progesterone (1 µg/mL)	2-8 °C
<input type="checkbox"/> Diluent 22	≤-10 °C
<input type="checkbox"/> Human Progesterone Calibrator (Set of 8 vials <sup>2</sup> )	-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

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

<sup>2</sup> 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  $\mu$ L/well, 100  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ L/well of Detection Reagent (solution of Diluent 22 containing diluted SULFO-TAG progesterone).
2. Add 25  $\mu$ L/well Calibrator or sample and incubate at room temperature with shaking for 1 hour.
3. Prepare SECTOR<sup>®</sup> instrument such that the plate can be read immediately following Read Buffer addition.
4. Wash plates 3 times with PBS.
5. Add 150  $\mu$ 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.*

