MSD® MULTI-ARRAY Assay System

Human Luteinizing Hormone (LH) Custom Kit

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MSD Toxicology Assays

Human Luteinizing Hormone (LH) Custom Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

MSD Customer Service

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MSD Scientific Support

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Principle of the Assay

MSD Toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human Luteinizing Hormone (LH) is a sandwich immunoassay. MSD provides a plate pre-coated with streptavidin, and the user adds capture antibody conjugated to biotin. The user then adds the sample and a solution containing the detection antibody conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Biotin-conjugated capture antibody binds to the streptavidin immobilized on the working electrode surface; analyte in the sample binds to the capture antibody and the recruitment of the labeled detection antibody by bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

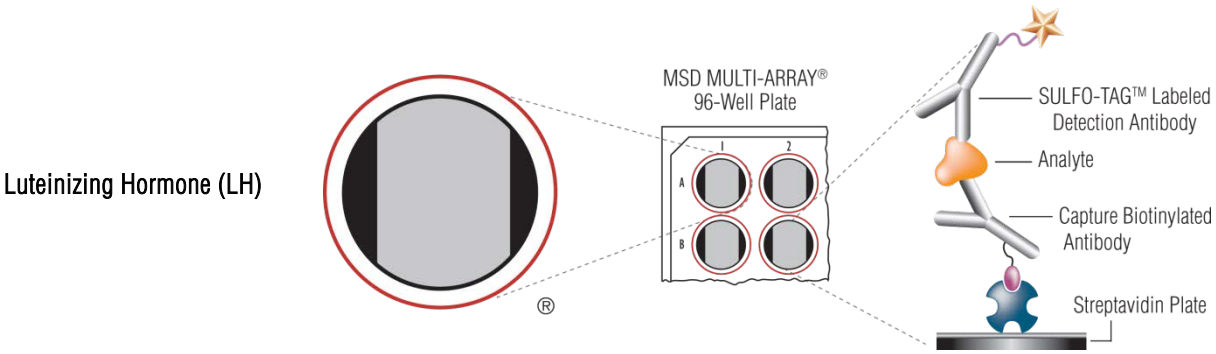


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Reagent	Storage
MSD GOLD™ 96-Well Streptavidin SECTOR® Plate	2–8°C
Anti-hu Luteinizing Hormone (LH) Biotinylated Capture Antibody (50X)	2–8°C
SULFO-TAG Anti-hu Luteinizing Hormone (LH) Antibody (50X)[1]	2–8°C
Luteinizing Hormone (LH) Calibrator [2]	≤-70°C
Diluent 23	≤-10°C
Diluent 22	≤-10°C
Blocker A Kit	RT
Read Buffer T (4X)	RT

[1]SULFO-TAG–conjugated detection antibodies should be stored in the dark.
[2]The calibrator in this kit is derived from human source material that has been tested and found to be negative for HIV-1 and 2, Hepatitis B, Hepatitis C. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing serial dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1,000 rpm.
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the material safety data sheet (MSDS), which can be obtained from MSD Customer Service.

Reagent Preparation

Bring all reagents to room temperature.

> **Important:** Upon first thaw, separate diluents into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Capture Antibody Solution

The biotin-conjugated capture antibody is provided as a 50X stock solution. The final concentration of the working capture antibody solution should be at 1X. For each plate used, add 60 µL of the stock capture antibody to 2.94 mL of Diluent 23.

Prepare Calibrator Dilutions

MSD supplies calibrator for the Human Luteinizing Hormone (LH) Kit at the concentration of the highest calibrator. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the calibration curve solutions.

Calibrator	Human Luteinizing Hormone (LH) (mIU/mL)	Dilution Factor
Calibrator-01	110	
Calibrator-02	28	4
Calibrator-03	6.9	4
Calibrator-04	1.7	4
Calibrator-05	0.43	4
Calibrator-06	0.11	4
Calibrator-07	0.027	4
Calibrator-08	0	n/a

To prepare 7 calibration solutions plus a zero calibrator blank for up to 4 replicates:

1. Use undiluted stock calibrator as Calibrator-01.
2. Prepare the next calibrator by transferring 50 µL of Calibrator-01 to 150 µL of Diluent 23. Mix well. Repeat 4-fold serial dilution 5 times to generate 7 calibrators.
3. Use Diluent 23 as the blank.

Avoid refreezing Human Luteinizing Hormone (LH) Calibrator stock.

Samples

Human serum and plasma samples do not normally require dilution. If results fall outside the detectable range of the assay, you may dilute samples in Diluent 23.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution used in the assay is a 1X solution. Prepare the detection antibody solution immediately prior to use.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Luteinizing Hormone (LH) Antibody
- ❑ 2.94 mL of Diluent 23

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Protocol

1. **Block plate.** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 30 minutes.

2. **Wash and Add Capture Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of capture antibody solution per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 30 minutes.

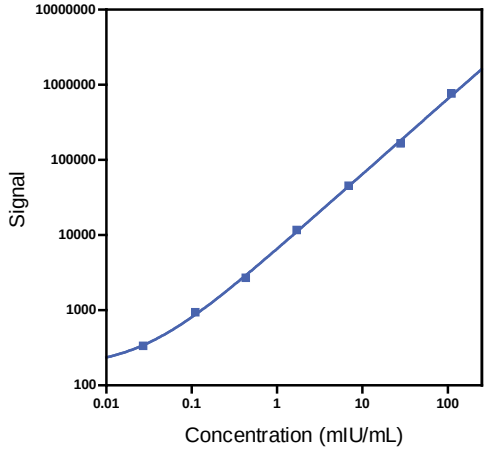
3. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of PBS-T. To each well, add 25 µL of Diluent 22 followed by 25 µL of sample, calibrator, or control. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

4. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

5. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Typical Data

The following calibration curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates of calibrators.



Luteinizing Hormone (LH)		
Conc. (pg/mL)	Average Signal	%CV
0	103	9.0
0.027	336	8.6
0.11	942	3.5
0.43	2,708	1.5
1.7	11,715	2.6
6.9	45,497	2.1
28	166,022	4.2
110	769,035	1.4

Summary Protocol

Human Luteinizing Hormone (LH) Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Luteinizing Hormone (LH) assays.

Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare capture antibody solution by diluting the stock capture antibody 50-fold in Diluent 23.

Prepare 7 calibration solutions using the supplied calibrator:

- The stock calibrator is at the concentration of the highest calibrator.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 23.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Block Plate

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with shaking for 30 minutes.

Step 2: Wash and Add Capture Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X capture antibody solution.

Incubate at room temperature with shaking for 30 minutes.

Step 3: Wash and Add Sample

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of Diluent 22 followed by 25 µL/well of sample (calibrators, controls, or unknowns).

Incubate at room temperature with shaking for 2 hours.

Step 4: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with shaking for 2 hours.

Step 5: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Read plate on MSD instrument.

Plate Diagram