

Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Human Luteinizing Hormone (LH) Assay Kit



1-Plate Kit	K151ETC-1
5-Plate Kit	K151ETC-2
25-Plate Kit	K151ETC-4

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MSD Toxicology Assays

Human Luteinizing Hormone (LH) Assay Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

ordering information

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Introduction

introduction

Luteinizing Hormone (LH) (Human) is produced in the pituitary and participates in regulation of testosterone and estrogen levels. LH is a glycoprotein hormone. Whereas the alpha subunit of this glycoprotein hormone is common to LH, FSH and TSH, it is the unique beta subunit that confers biological specificity. The MSD assay reagents react with the LH beta subunit without cross-reactivity to the other hormones. Biologically, decreased LH can lead to infertility in both the male and female. Luteinizing hormone release from the pituitary is under the control of luteinizing hormone-releasing hormone from the hypothalamus, a brain area that communicates with the brain and nervous system when to initiate or increase production of hormones. LH levels are often evaluated in the patient or the enrollee in clinical trials as part of a battery of tests to evaluate pituitary function involving control of the ovaries or testes.

Principle of the Assay

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. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Human Luteinizing Hormone (LH) Assay detects luteinizing hormone in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with luteinizing hormone antibody. The user adds the sample and a solution containing the labeled detection antibody—Anti-hLuteinizing Hormone (LH) labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Luteinizing hormone in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR[®] instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of luteinizing hormone present in the sample.

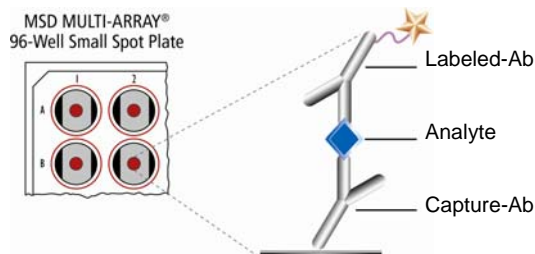


Figure 1. Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K151ETC-1	K151ETC-2	K151ETC-4
MULTI-ARRAY [®] 96-well Human Luteinizing Hormone (LH) Plate L451ETA-1	2-8°C	1 plate	5 plates	25 plates
SULFO-TAG [™] Anti-hLuteinizing Hormone (LH) Antibody ¹	2-8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Luteinizing Hormone (LH) Calibrator ² 110 mIU/mL	≤ -70°C	1 vial (200 µL)	5 vials (200 µL ea)	25 vials (200 µL ea)
Diluent FC R50BC-8 (1.5 mL) R50BC-4 (15 mL)	-20°C	1 vial (1.5 mL)	1 bottle (15 mL)	5 bottles (15 mL ea)
Diluent F R50BB-8 (8 mL) R50BB-4 (40 mL)	-20°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)



Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline (PBS) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

² The Calibrator in this kit is derived from human source material which has been tested and found to be negative for HIV-1 and 2, Hepatitis B, and Hepatitis C. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

V Safety

s a f e t y

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

VI Reagent Preparation

r e a g e n t p r e p a r a t i o n

Bring all reagents to room temperature.

Important: Upon first thaw, separate Diluent F into aliquots appropriate to the size of your assay needs. Diluent F can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay. Diluent FC may be refrozen twice.

Prepare Calibrator and Control Solutions

Calibrator for the Human Luteinizing Hormone (LH) Assay is supplied at the concentration of the highest Calibrator. For the assay, an 8-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Luteinizing Hormone (LH) (mIU/mL)	Dilution Factor
STD-01	110	
STD-02	27.50	4
STD-03	6.875	4
STD-04	1.719	4
STD-05	0.430	4
STD-06	0.107	4
STD-07	0.027	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 3 replicates:

- 1) Calibrator for the Human Luteinizing Hormone (LH) Assay is supplied at the concentration of the highest Calibrator. Therefore no dilution is required for top of the curve.
- 2) Prepare the next Calibrator by transferring 50 μ L of the undiluted Calibrator to 150 μ L of Diluent FC. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) Reserve 150 μ L of Diluent FC to be used as zero calibrator.

Calibrators should be prepared no more than 20 minutes before use. Calibrators should be stored at 4°C (for up to 4 hours) if not used immediately. Avoid repeated freeze-thawing of Human Luteinizing Hormone (LH) Calibrator stock.

Dilution of Samples (optional)

For human serum and plasma, no dilution is necessary. If samples fall outside of the detectable range of the assay, dilute in Diluent FC.

Prepare Detection Antibody Solution

The Detection Antibody is provided as a 50X stock of Anti-hLuteinizing Hormone Antibody. The working Detection Antibody Solution should contain 1X as final concentration. For each plate used, dilute 60 μ L of the stock Detection Antibody stock into a final volume of 3 mL of Diluent F.

Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of stock Read Buffer T (4X) to 10 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody as shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

- 1. Addition of Sample or Calibrator:** First, Dispense 25 μ L of Diluent F into each well. Then, dispense 25 μ L of sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 2. Addition of the Detection Antibody Solution:** Dispense 25 μ L of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 3. Wash and Read:** Wash the plate 3X with PBS. Add 150 μ L of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

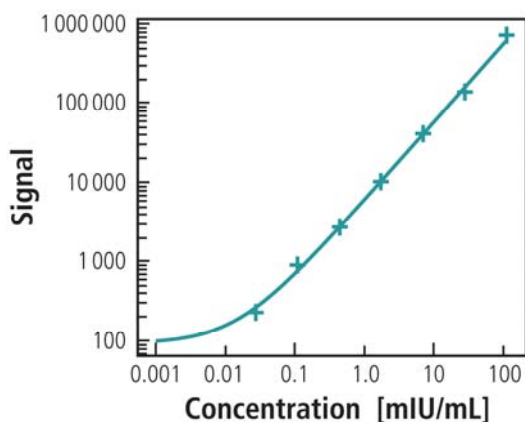
analysis of results

The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



Conc. (mIU/mL)	Mean	%CV
0	102	17
0.027	231	3.4
0.107	909	1.0
0.430	2749	1.5
1.719	10424	2.6
6.875	40938	1.4
27.50	137231	18
110	721718	6.4

X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

Luteinizing Hormone (LH) (mIU/mL)	
LLOD	0.006
LLOQ	0.055
ULOQ	110

XI Precision

precision

A multi-day, multi-plate study over 15 plates was performed to show reproducibility. In addition to the standard curves, control samples of a high, mid, and low levels of LH were measured on each plate. Each sample was run in triplicate. The average intra-plate %CV and inter-day %CV of the concentrations are shown below.

	Control	Plates	Average Conc. (mIU/mL)	Intra-plate	Inter-day
				Average % CV	%CV
LH	High	15	84.0	7.0	9.3
	Mid	15	31.2	7.4	9.8
	Low	15	6.24	5.8	10.7

XII Spike Recovery

Spike Recovery

Both male and female human serum samples were spiked with Human LH Calibrator at multiple levels throughout the range of the assay. All samples displayed good recovery and acceptable %CV for all spike levels. Results from 6 serum samples are shown in the table.

% Recovery = measured / expected x 100

Human Serum Sample	Spike Level (mIU/mL)	Concentration (mIU/mL)	Concentration %CV	% Recovery
Male 1	0	3.9	6.7	
	15	17.5	5.8	92
	20	32.8	10.8	97
	60	62.1	9.6	97
Male 2	0	3.7	3.1	
	15	15.6	13.2	83
	20	29.6	11.3	88
	60	53.1	5.4	83
Male 3	0	5.3	3.9	
	15	19.1	8.1	94
	20	33.8	2.2	96
	60	60.8	6.2	93
Male 4	0	5.4	5.8	
	15	18.2	13.5	89
	20	34.7	10.8	98
	60	58.3	7.8	89
Male 5	0	4.8	8.6	
	15	18.8	7.9	95
	20	31.4	8.2	90
	60	65.0	4.4	100
Female 6	0	3.8	2.9	
	15	15.7	9.6	84
	20	28.2	11.3	83
	60	63.0	2.0	99

XIII Linearity

linearity

Multiple human serum samples were assayed at 2, 4, 8, and 16-fold dilutions to measure linearity. Samples with high, mid and low level of LH were used to show linearity across the range of the assay. Linearity in five representative female serum samples is shown below. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

Human Serum Sample	Fold Dilution	Concentration (mIU/mL)	Concentration %CV	% Recovery
1	1	29.16	3.2	
	2	30.87	1.4	106
	4	30.25	15.2	98
	8	32.51	2.8	107
	16	33.36	7.3	103
2	1	53.87	10.7	
	2	54.84	8.2	102
	4	54.00	6.8	98
	8	48.31	2.2	89
	16	52.97	3.7	110
3	1	5.00	6.1	
	2	5.22	0.9	104
	4	5.62	0.6	108
	8	5.99	1.7	107
	16	5.82	4.2	97
4	1	3.17	1.8	
	2	3.16	7.8	100
	4	3.68	13.5	116
	8	3.45	1.8	94
	16	3.75	2.8	109
5	1	2.36	6.8	
	2	2.26	13.8	96
	4	2.20	15.8	97
	8	2.18	16.5	99
	16	2.14	6.6	98

XIV Samples

s a m p l e s

Normal human serum samples from 10 females and 5 males were run neat on the Human LH assay. The median levels and ranges for concentration and %CV for each sex are presented in the table below.

		Endogenous Levels of Human Luteinizing Hormone			
		Concentration (mIU/mL)		%CV	
# Samples		Median	Range	Median	Range
Female	10	5.2	2.2 – 55.2	11.4	3.4 – 19.1
Male	5	4.5	3.4 – 5.5	4.0	1.7 – 15.9

XV Calibrator

c a l i b r a t o r

Luteinizing hormone is purified from human pituitary extract. The purified sample is calibrated against and internal control and diluted to a final concentration of 110 mIU/mL to make the Human Luteinizing Hormone (LH) Calibrator.

Caution: *Human Luteinizing Hormone (LH) Calibrator is of human origin. Take appropriate precautions when handling this reagent.*

XVI References

r e f e r e n c e s

1. Jamnongjit M, Hammes SR. (2006) Ovarian Steroids: The Good, the Bad, and the Signals that Raise Them. Cell Cycle June;5(11):1178-1183
2. Brawer MK. (2004) Challenges with luteinizing hormone-releasing hormone agonists: flare and surge. Rev Urol. 6 Suppl 7:S12-8

Summary Protocol

MSD 96-well MULTI-ARRAY Human Luteinizing Hormone (LH) Assay Kit

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

Step 1 : Sample and Reagent Preparation

Bring appropriate diluents and plates to room temperature

If necessary, samples should be diluted in Diluent FC.

Prepare an 8-point standard curve using supplied calibrator:

- The Calibrator is supplied at the concentration of the highest Calibrator therefore no dilution is required for top of the curve.
- Dilute the stock Calibrator in Diluent FC to perform a series of 4-fold dilution steps and a no calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-hLuteinizing Hormone Antibody (LH) to 1X in 3.0 mL of Diluent F per plate.

Prepare 20 mL of 2X Read Buffer T by diluting MSD Read Buffer T (with surfactant), 4X with deionized water.

Step 2 : Add Sample or Calibrator

Dispense 25 μ L/well Diluent F.

Dispense 25 μ L/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3 : Add Detection Antibody Solution

Dispense 25 μ L/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Read Plate

Wash plate 3X with PBS.

Dispense 150 μ L/well 2X Read Buffer T.

Analyze plate on SECTOR instrument.

