Mouse Luteinizing hormone (LH) ELISA Kit

Catalog NO.: RK15292

version: 2.0

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

Introduction

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of LH in mouse serum, plasma, cell culture supernatants, tissue homogenates and other biological fluids.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Mouse LH has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LH present is bound by the immobilized antibody. After washing away any unbound substances, and then a detection antibody specific for LH is added to the wells and binds to the combination of capture antibody LH in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate solution is added to the wells and color develops in proportion to the amount of LH bound in the initial step. The color development is stopped and the absorbance is measured.

Material Provided & Storage Conditions

Unopened kits can be stored at 2-8° C for 1 year, and opened products must be used within 1 month.

Part	Size	Cat. No.	Storage of opened/reconstituted material
Mouse LH Microwell Plate Coated	8×12	RZ21165	Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8° C for 1 month.
Mouse LH Standard	6 ×1 mL	RZ21166	It is not recommended to use again after redissolving.
FITC -Antibody	1 x 6 mL	RZ21167	Store at 2-8° c for 1 month *
HRP Conjugate	1 x 11 mL	RZ21168	Store at 2-8° c for 1 month *

Wash Buffer(20x)	1 ×15 mL	RM00026	Store at 2-8° c for 1 month *
TMB Substrate A	1 ×7 mL	RM00027	
TMB Substrate B	1 ×7 mL	RM00027	
Stop Solution	1 ×7 mL	RM00028	
Plate Sealers	4 Strips		
Specification	1		

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
4. Stop Solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
8. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all diluents.
9. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.
10. Reuse of dissolved standard is not recommended.

11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. This assay is designed to eliminate interference by other factors present in biological samples.
17. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
18. The 48T kit is also suitable for the specification.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatant: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Tissue homogenates: The preparation of tissue homogenates varies depending upon tissue type. Tissues are rinsed in ice-cold PBS to remove excess blood thoroughly and weigh before

homogenization. Mince the tissues to small pieces and homogenized them in fresh lysis buffer with a glass homogenizer on ice or using Micro Tissue Grinders. Different lysis buffer should be chosen based on subcellular location of the target protein (e.g. 1mL lysis buffer is added in 200mg tissue sample). The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates are centrifuged for 5 minutes at 10,000 × g. Collect the supernatants and assay immediately or aliquot and store at ≤ -20 ° C.

Other biological fluids: Centrifuge samples for 20 minutes at 1,000 × g. Collect the supernatants and assay immediately or store samples in aliquot at -20° C or -80° C for later use. Avoid repeated freeze-thaw cycles.

Note: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Standard -Set concentration according to the following below:

Standard	S5	S4	S3	S2	S1	S0
mIU/mL	50	25	9	3	1	0

Wash solution (20X)- dilute the wash solution at 1:20 with double steam or deionized water before use.

Sample preparation

Different samples need to select the appropriate dilution multiple according to the specific situation. use the standard / sample dilution in the kit.

Cell supernatant - Because the cell supernatant samples are quite different due to the different experimental conditions, it is recommended to pretest the cell supernatant samples to determine the appropriate dilution multiple.

Serum / plasma - Samples tested, recommended stock solution.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal properly.
3. Without any liquid added to the blank wells, 50 μL of standard or samples were added to the other wells, then 50 μL FITC of labeled antibodies, mixed, blocked the plate wells with the provided sealed plate membrane, and incubated at 37℃ for 1 hour.
4. Add wash buffer 350 μL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
5. The enzyme conjugate (100 μL / well) was added to each well and covered with a new plate membrane and incubated at 37℃ for 0.5 hours.
6. Preheat enzyme plate reade.

7. Add wash buffer 350 μL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
8. Add 50 μL of chromogenic substrate A and chromogenic substrate B to the well and mix well with shock. Incubation was performed in the dark at 37℃ for 15 - 20 min.
9. The termination solution (50 μL / well) was added and immediately placed into the microplate reader to determine the OD of 450nm in each well within 5min. If the correction wavelength can be selected, set to 570 nm or 630 nm. And the 570 nm or 630 nm reads were subtracted from the 450 nm reads, which could correct and remove the OD of the non-chromogenic material to obtain more accurate detection results. If the correction wavelength cannot be selected, the obtained reading will be high, reducing the accuracy of the reading.

Assay Procedure Summary

Prepare the standard and reagents

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Add 50ul of standard or sample and 50 μL FITC to the wells to label antibodies

Incubate for 1 hours at 37℃, then wash 3 times

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Add 100ul of enzyme conjugate in each well

Incubate for 0.5 hour at 37℃, then wash 3 times

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Add 50ul of substrate solution A and 50ul, substrate solution B, and mix well with shock

Incubate for 15-20 min at 37℃ under dark condition

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Add 50ul Stop Solution

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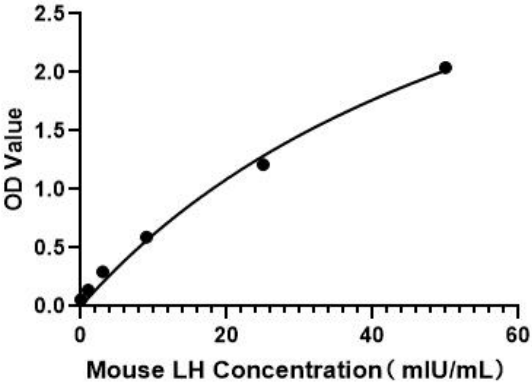
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four- parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the LH concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of LH assayed.

Detection Range

1-50 mIU/mL

Sensitivity

The minimum detectable dose (MDD) of LH typically less than 0.5 mIU/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of LH.
No significant cross-reactivity or interference between LH and analogues was observed.

Note:
Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between LH and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-plate Precision

3 samples with low, middle and high level LH were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level LH were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

Recovery

Matrices listed below were spiked with certain level of LH and the recovery rates were calculated by comparing the measured value to the expected amount of LH in samples.

Sample	Average Recovery (%)	Range (%)
Cell Culture Media(n=5)	98	88-108
Serum(n=5)	104	91-116

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of LH and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media(n=5)	Serum(n=5)
1:2	Average of Expected (%)	88	85
	Range (%)	82-95	82-92
1:4	Average of Expected (%)	90	87
	Range (%)	85-96	85-93
1:8	Average of Expected (%)	89	93
	Range (%)	81-96	85-97
1:16	Average of Expected (%)	90	92
	Range (%)	82-98	86-98

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.

	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

*For research purposes only. Not for therapeutic or diagnostic purposes.