LH-RH ELISA Kit

(For Human, Mouse, Rat, Porcine)

Enzyme Immunoassay for the quantification of LH-RH in blood, plasma, tissue and CSF extractions

Catalog number: ARG80913

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INTRODUCTION

LH-RH / Gn-RH is secreted and then cleaved to form the 10 aa luteinizing hormone-releasing hormone (LHRH, also known as gonadoliberin-1), and prolactin release-inhibiting factor (also known as GnRH-associated peptide 1). LHRH stimulates the release of luteinizing and follicle stimulating hormones, which are important for reproduction. Mutation in this gene are associated with hypogonadotropic hypogonadism. Alternatively spliced transcript variants have been described for this gene. [provided by RefSeq, Jul 2012]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of LH-RH in blood, plasma, tissue and CSF extractions.

This assay employs the competitive enzyme immunoassay technique. A secondary antibody has been pre-coated onto a microtiter plate and non-specific binding sites were blocked. Fc regions of primary antibodies specific for target peptides can bind to the secondary antibodies on microtiter plate. The Fab regions of primary antibodies are competitively bound by biotinylated peptide and targeted peptides in samples or standards. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The reaction is monitored by a color change which is readable at OD of 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards. The intensity of color development is inversely proportional to the amount of LH-RH in the samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Microtiter Plate	12 x 8 wells	4°C
20X assay buffer concentrate	50ml	4°C
Acetate Plate sealer (APS)	3 pieces	4°C
Primary antibody (Rabbit anti-peptide IgG)	1 vial	4°C
Standard peptide	1 vial	4°C
Biotinylated peptide	1 vial	4°C
Streptavidin-HRP	30 µl	4°C
Positive control	2 vials	4°C
Substrate Solution (TMB)	12 ml	4°C, ready for use
2N HCl	15 ml	4°C, ready for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Blood collection- Collect blood samples into tubes containing EDTA. Gently rock the tubes several times immediately to prevent coagulation. Transfer the blood from tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml blood) and gently rock for a few times to inhibit activity of proteinases. Centrifuge the blood at 1600 x g for 15 minutes at 4°C and collect plasma. Plasma kept at-80°C is stable for 1 month.

** In order to reduce background, peptide extraction of samples is highly recommended **

REAGENT PREPARATION

- **1X Assay Buffer**: Dilute 20X Assay buffer into distilled water to yield 1X Wash buffer. Keep 1X Assay buffer at 4°C. If crystals appear in 20X assay buffer, warm the buffer in warm water bath for 30 minutes or until crystals disappear. Mix well before use.
- **Primary antibody**: Reconstitute the Primary antibody vial with 5 ml of 1X assay buffer. Allow to sit for 5 minutes and mix well before use. Keep rehydrated solution at 4°C.
- **Biotinylated peptide**: Reconstitute the Biotinylated peptide vial with 5 ml of 1X assay buffer. Allow to sit for 5 minutes and mix well before use. Keep rehydrated solution at 4°C.
- **Positive control**: Reconstitute the Positive control vial with 200 µl of 1X assay buffer. Allow to sit for 5 minutes and mix well before use. Keep rehydrated solution at 4°C.
- **Standard peptide**: Reconstitute the Standard peptide vial with 1 ml of 1X assay buffer. Vortex. The concentration of this stock solution is 1000 ng/ml. Allow to sit for 10 minutes at RT. M ix well and spin down before use. Dilute standard solutions according to the table below and make serial dilutions of 25 ng/ml, 5 ng/ml, 1 ng/ml, 0.2 ng/ml and 0.04 ng/ml.

Tube No.	Standard volume	1X Assay Buffer	Concentrations (ng/ml)
Stock	1000 µl	0 µl	1000
1	25 µl of stock	975 µl	25
2	200 µl of Tube 1	800 µl	5
3	200 µl of Tube 2	800 µl	1
4	200 µl of Tube 3	800 µl	0.2
5	200 µl of Tube 4	800 µl	0.04

ASSAY PROCEDURE

1. Add 50 µl of 1X assay buffer as Total Binding. Two empty wells should be left as Blank.
2. Add 50 µl of prediluted peptide standards, 50 µl positive controls or 50 µl samples into corresponding wells. It is advisable to assay each condition in duplicates.
3. Add 25 µl of primary antibody into each well except the Blank wells.
4. Add 25 µl of Biotinylated peptide into each well except the Blank wells. It is not recommended to use a multi-channel pipette to load the primary antibody and biotinylated peptide.
5. Seal the microtiter plate with acetate plate sealer (APS). Incubate for 2 hours at RT. Orbital shaking at 300-400 rpm is recommended.
6. Centrifuge Streptavidin-HRP vial and pipette 12 µl of Streptavidin-HRP into 12ml of 1X Assay buffer. Vortex thoroughly. Prepare fresh.
7. Remove sealer from plate.
8. Aspirate each well and wash, repeating the process 1 times for a total 2 washes. Wash by filling each well with 1× Assay Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
9. Add 100 µl Streptavidin-HRP solution into each well.
10. Reseal the plate with sealer. Incubate for 1 hour at RT. Orbital shaking at 300-400 rpm is recommended.

11. Remove sealer from plate.
12. Wash as according to step 8.
13. Add 100 µl TMB substrate solution into each well.
14. Reseal the plate with sealer. Incubate for 1 hour at RT. Orbital shaking at 300-400 rpm is recommended. (Protect from light)
15. Remove sealer from plate.
16. Add 100 µl 2N HCl into all wells to stop the reaction.
17. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

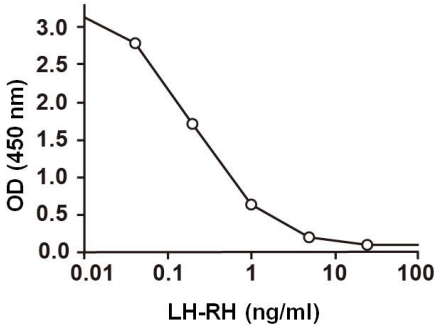
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Linear Range: 0.08 – 1.09 ng/ml

Sensitivity: 0.08 ng/ml

Cross Reactivity

This kit detects no cross-reactivity with the following factor:

LH-RH (Lamprey)

LH-RH (Salmon)

[D-Trp6, Des-Gly10]-LH-RH Ethylamide

[D-Ala6, Des-Gly10]-LH-RH Ethylamide

GAP (Human)

GRF (Human)

ACTH (Human)