

# hLH [I-125] IRMA KIT

(REF: RK-750CT)

The <sup>125</sup>I-hLH IRMA system provides direct quantitative *in vitro* determination of human Luteinizing Hormone (hLH) in human serum. hLH can be assayed in the range of 0.11-150 mIU/mL using 100 µl serum samples.

## Introduction

The human Luteinizing Hormone (Lutropin or LH) is a glycoprotein with a molecular weight of 30000, secreted by the adenohypophysis. Like other glycoprotein hormones (LH, TSH and HCG), hLH contains two different subunits, an α- and a β-chain, linked by noncovalent bonds. The primary structures of the α subunits of hLH and of those mentioned are virtually identical, whilst their β subunits are different. The β subunits are responsible for the immunological and biological specificity of these hormones.

The hLH synthesis and release is stimulated by the hypothalamic Gonadotrophin-Releasing Hormone (GnRH), whereas the ovarian steroids secreted from the corpus luteum control further secretions of hLH by negative feed-back.

The measurement of hLH concentrations is an important part of the investigation of disorders of the hypothalamic-pituitary-gonadal axis. It is recommended to measure both hLH and hFSH to discriminate between hypothalamic and pituitary dysfunction.

## Principle of the method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The <sup>125</sup>I labelled signal-antibody binds to an epitope of the LH molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 1-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hLH, the unknown concentration of hLH in patient samples can be determined.

## Contents of the kit

1. 1 bottle of TRACER (21 mL), ready to use, containing about 740 kBq <sup>125</sup>I-anti-hLH and capture anti-hLH in buffer with red dye 0.1 % NaN<sub>3</sub>.

2. 6 vials of STANDARDS (6 x 1.0 mL), containing (S<sub>1</sub>-S<sub>6</sub>) 0, 0.4, 2.0, 10, 40, 150 mIU/mL hLH (WHO 2<sup>nd</sup> IS 80/552 Int.Std.) in bovine serum with 0.1% NaN<sub>3</sub>.

3. 1 vial of CONTROL SERUM. 1 mL of lyophilized human serum with 0.1% NaN<sub>3</sub>. The concentration of the control serum is specified in the quality certificate enclosed. See *Preparation of reagents*.

4. 2 boxes of COATED TUBES, Ready to use. 2 x 50 reactive test tubes, 12x75 mm, packed in plastic boxes.

5. 1 bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN<sub>3</sub>. See *Preparation of reagents*.

Quality certificate  
Pack leaflet

## Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 and 2000 µL), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter

### Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-mL pipette)

## Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C) for 5 months. Frozen samples should be thawed and thoroughly mixed before assaying. Samples with a hLH concentration higher than that of the most concentrated standard should be diluted and reassayed.

## Preparation of reagents, storage

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. Upon dilution store at 2-8°C until expiry date of the KIT.

Add 1000 µL distilled water to the lyophilized control serum. Mix gently with shaking or vortexing (foaming should be avoided).

Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. Store at -20°C until expiry date of the KIT.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the KIT. The actual expiry date is given on the package label and in the quality certificate.

### CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

## Assay procedure

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S<sub>1</sub>-S<sub>6</sub>), control serum and samples.

2. Homogenize all reagents and samples by gentle mixing to avoid foaming.
3. Pipette 100 µL of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
4. Pipette 200 µL of tracer into each tube.
5. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (200 - 600 rpm recommended).
6. Incubate tubes for 1 hour, shaking at room temperature.
7. Add 2.0 mL of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside-down position place the rack on an absorbent paper for 2 minutes.
8. Return the tube-rack to an upright position, and repeat step-7 one more time.
9. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the LH concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microliters)

Tubes	Total	Standard	Control	Sample
Standard		100		
Control			100	
Sample				100
Tracer	200	200	200	200
Shake for 1 hour at room temperature				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

## Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T (\%) = \frac{S_{2-6} / C / M_x (\text{cpm}) - S_1 (\text{cpm})}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of hLH.

Determine the hLH concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Table 2. Typical assay data

Tubes	Mean cpm (n = 15)	B/T%	mIU/mL
T	293451	-	-
S1	121	0.04	0
S2	726	0.25	0.4
S3	3066	1.04	2
S4	14462	4.93	10
S5	55331	18.86	40
S6	174885	59.60	150
C	12274	4.18	8.3

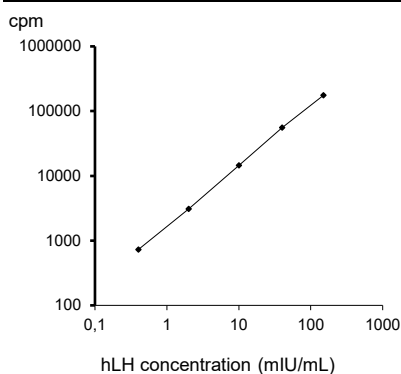


Figure 1: A typical standard curve (Do not use to calculate unknown samples)

## Characterization of the assay

### Typical assay parameters

#### Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

Limit of Blank (LoB): 0.05 mIU/mL  
 Limit of Detection (LoD): 0.09 mIU/mL  
 Limit of Quantitation (LoQ): 0.11 mIU/mL

#### Specificity

Cross-reaction with hFSH is 0.41% and with beta-hCG is 1.02%. No cross reactivity with hTSH, hGH and hPRL was detected for concentrations higher than physiological values.

#### Precision

5 patient samples were assayed in 15 replicates to determine intra-assay precision. Values obtained are shown below.

Sample	Number of replicates	Mean value	SD	CV %
1	15	52.3	0.39	0.7
2	15	29.9	0.28	1.0
3	15	6.6	0.1	1.4
4	15	1.4	0.03	2.2
5	15	0.2	0.02	9.3

#### Reproducibility

To determine inter-assay precision 6 patient samples were measured in duplicates in 15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Sample	Number of runs	Mean value	SD	CV %
1	15	0.21	0.03	12.1
2	15	6.5	0.2	3.1
3	15	29.2	0.91	3.1
4	15	51.8	1.45	2.8
5	15	1.44	0.06	4.1
6	15	8.4	0.34	4.0

#### Multisite precision

Multisite precision was calculated using 5 serum pools at different hLH concentrations, according to CLSI document EP05-A3. Results: Repeatability < 7%, Within laboratory precision < 7%, Reproducibility < 8%, for all concentrations evaluated.

#### Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of hLH. The average per cent recovery for 6 serum pools spiked with hLH at 3 levels was: 95.6% (mean).

#### Linearity

The linearity was evaluated according to CLSI EP06-A guideline, using the polynomial method. The method was found linear from 0.05 mIU/mL to 192.8 mIU/mL, within 10% error in this interval.

#### Interference:

Samples containing up to 190 µmol/L bilirubin, 11.0 mmol/L triglycerides, 12.6 g/L haemoglobin and 100 ng/mL biotin do not affect the concentration of hLH assayed.

#### Expected Values

male: 1.9 - 9.4 mIU/mL  
 female:  
 ovulatory peak: 25-94 mIU/mL  
 pre- and postovulatory: 0.7-9.0 mIU/mL  
 postmenopausal: 13-80 mIU/mL  
 It is recommended that each laboratory determine a reference range for its own patient population.

#### Limitations

- The reagents supplied in this kit are optimized to measure hLH levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Haemolyzed and lipemic specimens may give false values and should not be used.
- The KIT has no "high-dose hook" effect with hLH levels up to 1000 mIU/mL. Samples expected to have concentrations greater than the highest standard should be diluted with the S<sub>1</sub> (0 mIU/ml) and reassayed.
- The results of this assay should be used in conjunction with other pertinent clinical information.

#### Procedural notes

**Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

**Addition of wash buffer.** For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In

lack of this tool a large-volume syringe attached to a repeating pipette can be used.

**Additional information:** Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

## Precaution and warnings

### Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

### Potentially infectious materials

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1), Hepatitis B surface Antigen (HBsAg) and Treponema antibody.











Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*. Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

### Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

### Storage and shelf life

Store this product at a temperature of 2-8°C  
 Shelf-life: 60 days from availability.

	Use by date	<b>CONTROL</b>	Control
	Batch code	<b>CAL</b>	Standard
	Caution, consult accompanying documents	<b>CT</b>	Coated tube
	Biological risk	<b>TRAC</b>	Tracer
	Consult instructions for use	<b>WASHB</b>	Wash buffer
	In vitro diagnostic medical device		Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
<b>REF</b>	Catalogue number		

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