



Read entire protocol before use.

# LH-Irma

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

## **I. INTENDED USE**

Immunoradiometric assay kit for the *in vitro* quantitative measurement of human Luteinizing Hormone (LH) in serum.

## **II. GENERAL INFORMATION**

A. Name: Bio-Line **LH-Irma** Kit  
B. Catalogue number: **BL-23-CT**: 100 tests  
C. Manufactured by: Bio-Line S.A.  
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## **III. CLINICAL BACKGROUND**

### **A. Biological Activity**

Both LH and FSH are secreted by the basophil cells of the anterior pituitary as a result of gonadotropin releasing hormone (GnRH) secretion from hypothalamic cells. In adults, LH and FSH hormones control gonadal functions; mainly gametogenesis and steroid secretion.


### **B. Clinical Application**

The measurement of LH and FSH concentrations in serum is essential for investigating fertility and especially disorders of the hypothalamic/pituitary/gonadal axis. The LHsp-IRMA is a one step assay which is specific for LH. This specific assay enables the measurement of LH concentrations in serum, irrespective of the presence of hCG from endogenous (pregnancy or ectopic tumor) or exogenous origin (*in vitro* fertilization program, with pregnyl injection).

#### IV. PRINCIPLES OF THE METHOD

The Bio-Line LH-IRMA is an immunoradiometric assay based on coated tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with <sup>125</sup>I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity.

#### V. REAGENTS PROVIDED

Reagents	Qty 100 tests	Qty 4x100 tests	Colour Code	Reconstitutio n		
 Tubes coated with anti LH (monoclonal antibodies)	2 x 50	8 x 50	blue	Ready for use		
<table border="1" data-bbox="65 712 188 757"><tr><td>Ab</td><td><sup>125</sup>I</td></tr></table> Anti-LH- <sup>125</sup> I (monoclonal antibodies) in TRIS-HCl Buffer with bovine serum albumin, sodium azide (<0.1 %) and inert red dye	Ab	<sup>125</sup> I	1 vial 5.5 ml 700 kBq	4 vials 5.5 ml 4x700 kBq	red	Ready for use
Ab	<sup>125</sup> I					
<table border="1" data-bbox="65 904 199 949"><tr><td>CAL</td><td>0</td></tr></table> Zero Calibrator in bovine serum with thymol	CAL	0	1 vial lyophil.	2 vials lyophil.	yellow	Add 2 ml distilled water
CAL	0					
<table border="1" data-bbox="65 1025 199 1070"><tr><td>CAL</td><td>N</td></tr></table> Calibrators 1-6 in bovine serum with thymol (see exact values on vial labels)	CAL	N	6 vials lyophil.	12 vials lyophil.	yellow	Add 1 ml distilled water
CAL	N					
<b>WASH SOLN CONC</b>  Wash solution (TRIS-HCl)	1 vial 10 ml	4 vials 10 ml	brown	<b>Dilute</b> 70x with distilled water (use a magnetic stirrer).		
<table border="1" data-bbox="65 1397 261 1442"><tr><td>CONTROL</td><td>N</td></tr></table> Controls 1 and 2 in human serum with thymol	CONTROL	N	2 vials lyophil.	4 vials lyophil.	silver	Add 0.5 ml distilled water
CONTROL	N					

Note: 1. Use the zero calibrator for sera dilutions.  
2. 1 mIU of the calibrator preparation is equivalent to 1 mIU of 2<sup>nd</sup> IRP 80/552.

#### VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- Distilled water
- Pipettes for delivery of: 50 µl, 100 µl, 500 µl, 1 ml and 2 ml. (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Tube shaker
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional).
- Any gamma counter capable of measuring <sup>125</sup>I may be used (minimal yield 70%).

#### VII. REAGENT PREPARATION

- Calibrators** : Reconstitute the zero calibrator with 2 ml distilled water and the other calibrators with 1 ml distilled water..
- Controls** : Reconstitute the controls with 0.5 ml distilled water.

- Working Wash solution** : Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

#### VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 3 days at 2-8°C.  
For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

#### IX. SPECIMEN COLLECTION AND PREPARATION

- Serum must be kept at 2 – 8°C.
- If the test is not run within 24 hours, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

#### X. PROCEDURE

##### A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

##### B. Procedure

- Label coated tubes in duplicate for each calibrator, control and sample. For determination of total counts, label 2 normal tubes.
- Briefly vortex calibrators, controls and samples and dispense 100 µl of each into the respective tubes.
- Dispense 50 µl of anti-LH-<sup>125</sup>I tracer into each tube, including the uncoated tubes for total counts.
- Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
- Incubate for 1 hour at room temperature on a tube shaker (700 rpm).
- Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
- Wash the tubes with 2 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
- Aspirate (or decant) the content of each tube (except total counts).
- Wash again the tubes with 2 ml Wash Solution (except total counts) and aspirate (or decant).
- After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
- Count the tubes in a gamma counter for 60 seconds.

#### XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations.
- On semi logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of LH (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
- Read the concentration for each control and sample by interpolation on the calibration curve.
- Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

## XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

LHsp-IRMA		cpm	B/T (%)
Total count		246440	100
Calibrator	0.0 mIU/ml	207	0.1
	1.8 mIU/ml	533	0.2
	3.5 mIU/ml	1165	0.5
	9.9 mIU/ml	3821	1.6
	30.0 mIU/ml	12920	5.2
	97.0 mIU/ml	50053	20.3
	194.0 mIU/ml	93732	38.0

## XIII. PERFORMANCE AND LIMITATIONS

### A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average counts at zero binding, was 0.2 mIU/ml.

### B. Specificity

Cross-reactive hormones were added to a low and to a high LH value calibrator. The apparent LH response was measured.

added Hormone	LHsp CAL 1 mIU/ml	LHsp CAL 5 mIU/ml
-	1.8	97
FSH 300 mIU/ml	1.6	87
hCG 300000 mIU/ml	2.4	100
TSH 300 µIU/ml	3.6	85

### C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	<X> ± S.D. mIU/ml	CV (%)	Serum	N	<X> ± S.D. (mIU/ml)	CV (%)
A	10	6.6 ± 0.3	3.9	C	20	5.9 ± 0.5	8.0
B	10	49.6 ± 0.7	1.4	D	20	56.7 ± 1.9	3.4

### D. Accuracy

#### RECOVERY TEST

Sample	Added LH (mIU/ml)	Recovered LH (mIU/ml)	Recovery (%)
1	0.5	0.7	130
	1.5	1.8	117
	5	4.5	89
	14	12.7	90
	46	46.1	100
2	0.5	0.7	140
	1.5	1.3	87
	5	4.7	94
	14	12.9	92
	46	43.8	95

#### DILUTION TEST

Sample	Dilution	Theoretical Concent. (mIU/ml)	Measured Concent. (mIU/ml)
1	1/1	-	79.7
	1/2	39.9	41.1
	1/4	19.9	19.9
	1/8	10.0	10.1
	1/16	5.0	5.3
	1/32	2.5	2.2
2	1/1	-	121.0
	1/2	60.5	53.0
	1/4	30.3	27.9
	1/8	15.1	14.9
	1/16	7.6	7.8
	1/32	3.8	3.8

Samples were diluted with zero calibrator.

### E. Time Delay

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to coated tubes.

#### TIME DELAY

	0'	30'
Sample 1 (mIU/ml)	2.2	2.3
Sample 2 (mIU/ml)	4.8	5.4
Sample 3 (mIU/ml)	53.6	54.6

### F. Hook effect

A sample spiked with LH up to 1700 mIU/ml gives a signal above the highest calibrator concentration.

## XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

## XV. REFERENCE INTERVALS

The values provided below are given only for guidance; each laboratory should establish its own normal range of values.

The range is expressed as 2.5% to 97.5% percentiles.

Identification	Number of subjects	Mean (mIU/ml)	Range (mIU/ml)
<b>Children</b>	27	2.3	0.1 – 8.9
<b>Adult males</b>	69	2.7	1.0 – 5.3
<b>Women</b>			
· Ovulatory cycles			
- Follicular phase (day -12 to -6)	34	4.3	0.8 – 10.4
	49	19.6	2.9 – 41.1
- Ovulatory peak (day 0)	63	3.3	0.5 – 7.6
- Luteal phase (day +6 to +12)	53	31.2	14.4 – 52.8
· Postmenopausal			

## XVI. PRECAUTIONS AND WARNINGS

### Safety

For in vitro diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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## XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) CONTROLS ml
Calibrators (0-6) Samples, controls Tracer	- - 0.05	0.1 - 0.05	- 0.1 0.05
Incubation	1 hour at room temperature with shaking at 700 rpm		
Separation	-	aspirate (or decant)	
Washing solution	-	2.0	
Separation	-	aspirate (or decant)	
Washing solution	-	2.0	
Separation	-	aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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