

Read entire protocol before use.

Free β hCG-IRMA

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

Not be used for risk calculation of Trisomie 21

I. INTENDED USE

Immunoradiometric assay kit for the *in vitro* quantitative measurement of Free β human Chorionic Gonadotropin (β hCG) in serum.

II. GENERAL INFORMATION

- A. Name: Bio-Line **Free β hCG-IRMA** -CT Kit
- B. Catalogue number : **BL-51-CT**: 100 tests
- C. Manufactured by : Bio-Line S.A.
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III. CLINICAL BACKGROUND

A. Biological activities

The chorionic gonadotropic hormone is synthesised by the syncytiotrophoblast of the placenta all along the pregnancy and is released in the blood flow as soon as the 9th day following ovulation. The hCG has biologic characteristics similar to the LH. During pregnancy, this placental hormone stimulates the remaining corpus luteum that secretes oestrogen and progesterone for the first three months of the pregnancy. The hCG also stimulates developing placental elements which secrete the various steroid hormones, while the function of corpus luteum decreases, the level of oestrogen and of progesterone continues to increase as the placenta matures. In addition to that stimulating action on the luteal and placental tissue, the hCG by crossing the placenta is essential to differentiate the genital tractus of the foetus, which occurs around the 7th week of the pregnancy. The foetal hypophysis does not secrete gonadotropins until later stages of maturation. The hCG has also specific characteristics of FSH and TSH that is why most of the authors regard the hCG as the "ancestral" placental glycoprotein hormone having at the same time the three biologic hypophyseal hormones. The hCG of 37,900 Daltons comprises of two subunits, α and β bound in a non covalent way, as FSH, LH, TSH hormones. The hCG α -subunit of 14,900 Daltons is similar to the α -subunits constituting the hypophyseal hormones. The β -subunits specific to every glycoprotein hormone and gives their specific biologic activity. The chemical structures differ from one glycoprotein hormone to another, but for the β -subunits there is an antigenic continuity from one type to another. The hCG β -subunit is very similar to the LH β -subunit.

B. Clinical application

1. Diagnostic and monitoring test in pregnancy
hCG and its free subunits α and β appear in the serum and urine of pregnant women about 9 days following ovulation. The Free β hCG level then increases rapidly to reach a peak between the 8th and the 12th week.
2. Tumour marker test in trophoblastic tumours
Hydatiform moles and choriocarcinomas may secrete large amounts of native hCG and its two free subunits α and β into the peripheral blood circulation.
3. Tumour marker test in non-trophoblastic cancers
10 to 15 % of the breast, lung, and digestive tract cancers release hCG and/or either of its two constitutive subunits α and β .

IV. PRINCIPLES OF THE METHOD

A. Key feature


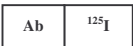


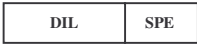


The Free β hCG IRMA has been developed in order to provide medical laboratories with an assay characterized by two dominant features :

- A 100 % recognition with free β chain to guarantee detection of ectopic tumour secreting predominantly free β chain rather than native hCG.
- A pregnancy test that provides a precise quantitative measurement of β hCG Free level in serum when using 1 hour incubation protocol without cross reaction with dimeric hCG.

B. Principle of the test

The Bio-Line β hCG-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 ^{125}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	Quantity 100 tests	Colour Code	Reconstitution
 Tubes coated with anti β hCG (monoclonal antibodies)	2 x 50	black	Ready for use
 Ab ^{125}I Anti- β hCG ^{-125}I (monoclonal antibodies) in Phosphate Buffer with bovine serum albumin, sodium azide (< 0.1 %) and inert red dye	1 vial 22 ml 750 kBq	red	Ready for use
 CAL 0 Zero Calibrator in human serum with thymol	1 vial lyophil.	yellow	Add 0.5 ml distilled water
 CAL N Calibrators 1-7 in human serum with thymol (see exact values on vial labels)	7 vials lyophil.	yellow	Add 0.5 ml distilled water
 DIL SPE Specimen diluent: Phosphate buffer with bovine serum albumin and azide (< 0.1%)	1 vial 22 ml	black	Ready for use
 INC BUF Incubation Buffer: Phosphate buffer with bovine serum albumin and azide (< 0.1%)	1 vial 22 ml	black	Ready for use
Wash Solution concentrate Wash solution (TRIS-HCl)	2 vials 10 ml	brown	Dilute 70 x in distilled water (use a magnetic stirrer).
 CONTROL N Controls 1 and 2 in human serum with thymol	2 vials lyophil.	silver	Add 0.5 ml distilled water

Note: 1. Use the specimen diluent for sera dilutions.

2. 1 mIU of the calibrator preparation is equivalent to 1 mIU of the 3rd IRP 75/551.

VI. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Pipettes for delivery of: 50 μl , 200 μl , 500 μl and 1 ml. (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer

4. Magnetic stirrer
5. Tube shaker (600 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional).
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- A. Calibrators: Reconstitute the calibrators with 0.5 ml distilled water..
- B. Controls: Reconstitute the controls with 0.5 ml distilled water.
- C. 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 7 days at 2-8°C.
For longer storage periods, aliquots should be made and kept at -20°C for 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

1. Label coated tubes in duplicate for each calibrator, control and sample. For determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, samples and controls and dispense 50 μl of each into the respective tubes.
3. Dispense 200 μl of Incubation Buffer into each tube except total counts.
4. Incubate for 30 min at room temperature with continuous shaking (600 rpm).
5. 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.
6. Wash the tubes twice with 3 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
7. Aspirate (or decant) the content of each tube (except total counts).
8. After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
9. Dispense 200 μl of anti- β hCG- ^{125}I tracer into each tube, including the uncoated tubes for total counts.
10. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
11. Incubate for 30 min at room temperature with continuous shaking (600 rpm).
12. 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.
13. Wash the tubes with 3 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
14. Aspirate (or decant) the content of each tube (except total counts).

15. Let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
16. Count the tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semi-logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of β hCG (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. 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.

β hCG-IRMA		cpm	B/T (%)
Total count		230469	100
Calibrator	0 mIU/ml	299	0.1
	0.29 mIU/ml	1196	0.5
	0.96 mIU/ml	3084	1.3
	2.8 mIU/ml	8790	3.8
	8.8 mIU/ml	25501	11.1
	28 mIU/ml	68274	29.6
	56 mIU/ml	108661	47.1
	93 mIU/ml	146141	63.4

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 0.03 mIU/ml.

B. Specificity

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

added Hormone	β hCG CAL 1 mIU/ml	β hCG CAL 6 mIU/ml
-	0.29	56
FSH 300 mIU/ml	0.28	58
TSH 300 μ IU/ml	0.25	58
LH 300 mIU/ml	0.31	60
β hCG 10000 ng/ml	46.8	86
hCG 500 ng/ml	0.8	55

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	$\langle X \rangle \pm$ S.D. (mIU/ml)	CV (%)	Serum	N	$\langle X \rangle \pm$ S.D. (mIU/ml)	CV (%)
A	10	1.42 \pm 0.10	7.3	C	20	1.32 \pm 0.08	5.8
B	10	10.7 \pm 0.3	2.5	D	20	8.9 \pm 0.3	3.6

D. Accuracy

RECOVERY TEST

Sample	Added β hCG (mIU/ml)	Recovered β hCG (mIU/ml)	Recovery (%)
A	0.44	0.38	87.2
	0.81	0.79	97.9
	3.66	3.88	106.0
	19.5	19.0	97.4
	37.3	36.7	98.4

DILUTION TEST

Sample	Dilution	Theoretical Concent. (mIU/ml)	Measured Concent. (mIU/ml)
1	1/1	-	7.73
	1/2	3.87	4.21
	1/5	1.55	1.31
	1/10	0.77	0.76
	1/20	0.39	0.43
2	1/1	-	30.62
	1/2	15.31	15.29
	1/5	6.12	6.13
	1/10	3.06	2.88
	1/20	1.53	1.51

Samples were diluted with specimen diluent.

E. Time Delay

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

TIME DELAY

	0' (mIU/ml)	10' (mIU/ml)	20' (mIU/ml)	30' (mIU/ml)
Serum 1	1.33	1.35	1.34	1.35
Serum 2	10.27	9.96	10.56	10.49

F. Hook effect

A serum sample with a concentration of 12800 mIU/ml β hCG 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 Practices

XV. REFERENCE INTERVALS

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

Identification	Number of subjects	Values (mIU/ml)
Normal subjects	33	< 0.1 mIU/ml

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.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) CONTROLS ml
Calibrators (0-7) Samples / Controls Incubation Buffer	- 0.2	0.05 - 0.2	- 0.05 0.2
Incubation	30 minutes at room temperature with continuous shaking		
Separation Washing solution Separation Washing solution Separation	- - - - -	aspirate (or decant) 3.0 aspirate (or decant) 3.0 aspirate (or decant)	
Tracer	0.2	0.2	0.2
Incubation	30 minutes at room temperature with continuous shaking		
Separation Washing solution Separation	- - -	aspirate (or decant) 3.0 aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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