

Read entire protocol before use.

PRL-Elisa

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

I. INTENDED USE

Immunoenzymetric assay for the *in vitro* quantitative measurement of human Prolactin (PRL) in serum and plasma.

II. GENERAL INFORMATION

- A. Name: Bio-Line Prolactin-Elisa kit
- B. Catalogue number: BL-25-E: 96 tests
- C. Manufactured by: Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
- D. For technical assistance or ordering information contact:
Tel: +32-2-736.62.18. Fax: +32-2-742.13.15.

III. CLINICAL BACKGROUND

A. Biological activities

Prolactin (PRL) is a polypeptide hormone (molecular weight 20,000 Da) secreted by the pituitary gland, which plays a key role in the development of the mammary gland, the production and secretion of milk and the control of male and female gonadal functions. Prolactin secretion is under hypothalamic control exerted directly by dopamine, several prolactin releasing factors (PRF) and perhaps VIP (vasoactive intestinal polypeptide) or a closely related peptide. TRH also acts directly at the pituitary level to stimulate prolactin release but its physiological role in the control of prolactin secretion has not been established yet. Several neuroendocrine factors, involving serotonergic or noradrenergic pathways are also involved in the control of prolactin secretion. The plasma concentration of prolactin increases in various physiological situations such as stress, pregnancy and lactation. Physiological levels fluctuate according to a nycthemeral rhythm, a significant rise being observed at night. Drugs with anti-dopamine activity (psychotropic agents) and ovulatory suppressants, increase prolactin secretion.

B. Clinical application


- *Prolactinoma* : Circulating prolactin levels are elevated in patients with a prolactin secreting pituitary adenoma. Amenorrhea and impotence are characteristic clinical symptoms in such cases.
- *Other pituitary diseases* : Increased prolactin levels are also observed in 5% to 20% of patients with acromegaly and when pituitary control by the hypothalamus is suppressed (pituitary stalk section). Decreased PRL levels may be observed in cases of complete destruction of the pituitary as in Sheehan's syndrome.
- *Galactorrhea and amenorrhea* : The measurement of the prolactin levels in serum is a useful test in the differential diagnosis of galactorrhea and amenorrhea.

IV. PRINCIPLES OF THE METHOD

The Bio-Line PRL-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MABs) directed against distinct epitopes of Prolactin. Calibrators and samples react with the capture monoclonal antibody (MAB 1) coated on microtiter well and with a monoclonal antibody (MAB 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAB 1 – human Prolactin – MAB 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the Prolactin concentration.

A calibration curve is plotted and PRL concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

V. REAGENTS PROVIDED

Reagents	96 tests Kit	Color Code	Reconstitution			
 Microtiterplate with 96 anti PRL (monoclonal antibodies) coated wells	96 wells	blue	Ready for use			
<table border="1" data-bbox="97 909 248 958"> <tr> <td>Ab</td> <td>HRP</td> </tr> </table> Conjugate: HRP labelled anti-PRL (monoclonal antibodies) in TRIS-HCl buffer with bovine serum albumin and thymol	Ab	HRP	1 vial 11 ml	red	Ready for use	
Ab	HRP					
<table border="1" data-bbox="102 1070 233 1111"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero calibrator in human serum and merthiolate (0.024%)	CAL	0	1 vial lyophilized	yellow	Add 2.0 ml distilled water	
CAL	0					
<table border="1" data-bbox="97 1205 233 1245"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrator N = 1 to 5 (see exact values on vial labels) in human serum and merthiolate (0.024%)	CAL	N	5 vials lyophilized	yellow	Add 0.5 ml distilled water	
CAL	N					
<table border="1" data-bbox="92 1346 285 1379"> <tr> <td>WASH</td> <td>SOLN</td> <td>CONC</td> </tr> </table> Wash Solution (Tris-HCl)	WASH	SOLN	CONC	1 vial 10 ml	brown	Dilute 200 x with distilled water (use a magnetic stirrer).
WASH	SOLN	CONC				
<table border="1" data-bbox="97 1440 261 1473"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum with thymol	CONTROL	N	2 vials lyophilized	silver	Add 0.5 ml distilled water	
CONTROL	N					
<table border="1" data-bbox="92 1563 285 1597"> <tr> <td>CHROM</td> <td>TMB</td> <td>CONC</td> </tr> </table> Chromogen TMB (Tetramethylbenzidine) in Dimethylformamide	CHROM	TMB	CONC	1 vial 1 ml	green	Dilute 0.2 ml into 1 vial of substrate buffer
CHROM	TMB	CONC				
<table border="1" data-bbox="81 1697 252 1742"> <tr> <td>SUB</td> <td>BUF</td> </tr> </table> Substrate buffer: H ₂ O ₂ in acetate / citrate buffer	SUB	BUF	3 vials 21 ml	white	Ready for use	
SUB	BUF					
<table border="1" data-bbox="76 1839 269 1883"> <tr> <td>STOP</td> <td>SOLN</td> </tr> </table> Stopping solution: H ₂ SO ₄ 1.8N	STOP	SOLN	1 vial 6 ml	black	Ready for use	
STOP	SOLN					

Note: 1. Use the zero calibrator for sample dilutions.

2. 1 ng of the calibrator preparation is equivalent to 30 ± 2 µIU MRC 3rd IRP 84/500.

VI. SUPPLIES NOT PROVIDED

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

- High quality distilled water
- Pipettes for delivery of: 25 µl, 50 µl, 100 µl, 200 µl, 500 µl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
- Washer for Microtiterplates
- Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (monochromatic reading)
- Optional equipment: The ELISA-AID™ necessary to read the plate according to polychromatic reading (see paragraph XI.A.) can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

VII. REAGENT PREPARATION

- Calibrators** : Reconstitute the zero calibrator with 2.0 ml distilled water and other calibrators with 0.5 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 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.
- Revelation Solution**: pipette 0.2 ml of the chromogen TMB into one of the vials of substrate buffer (H₂O₂ in acetate/citrate buffer). Extemporaneous preparation is recommended.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators and controls are stable for 1 week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum and plasma must be kept at 2 - 8°C.
- If the test is not run within 24 hours, storage in aliquots at -20°C is recommended. Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Serum or plasma (EDTA and heparin plasma) provides similar results.

$$Y(\text{serum}) = 0.88 \times (\text{EDTA plasma}) + 0.7 \quad r=0.96 \quad n=69$$

$$Y(\text{serum}) = 1.01 \times (\text{Heparin plasma}) - 9.0 \quad r=0.97 \quad n=69$$
- Do not use haemolysed samples.

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.
Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
Use a clean plastic container to prepare the Wash Solution.
In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
For the dispensing of the Revelation Solution and the Stop Solution avoid pipettes with metal parts.

High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section XIII paragraph E (Time delay).

Prepare a calibration curve for each run, do not use data from previous runs.

Dispense the Revelation Solution within 15 minutes following the washing of the microtiterplate.

During incubation with Revelation Solution, avoid direct sunlight on the microtiterplate.

B. Procedure

- Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
- Secure the strips into the holding frame.
- Pipette 25 µl of each Calibrator, Control and Sample into the appropriate wells.
- Pipette 100 µl of anti-PRL-HRP conjugate into all the wells.
- Incubate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
- Aspirate the liquid from each well.
- Wash the plate 3 times by:
 - Dispensing 0.4 ml of Wash Solution into each well
 - Aspirating the content of each well
- Pipette 200 µl of the freshly prepared revelation solution into each well within 15 minutes following the washing step.
- Incubate the microtiterplate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
- Pipette 50 µl of Stop solution into each well.
- Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section XI.

XI. CALCULATION OF RESULTS

A. Polychromatic Reading:

- In this case, the ELISA-AID™ software will do the data processing.
- The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
- A second reading is performed at 490 nm against the same reference filter.
- The ELISA-AID™ Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
- The principle of polychromatic data processing is as follows:
 - $X_i = OD$ at 450 nm
 - $Y_i = OD$ at 490 nm
 - Using a standard unweighted linear regression, the parameters A & B are calculated : $Y = A * X - B$
 - If $X_i < 3$ OD units, then X calculated = X_i
 - If $X_i > 3$ OD units, then X calculated = $(Y_i - B) / A$
 - A 4 parameter logistic curve fitting is used to build up the calibration curve.
 - The prolactin concentration in samples is determined by interpolation on the calibration curve.

B. Bichromatic Reading

- Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
- Calculate the mean of duplicate determinations.
- On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of PRL (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
- 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.

PRL-ELISA		OD units Polychromatic model
Calibrator	0 µIU/ml	0.068
	100 µIU/ml	0.142
	300 µIU/ml	0.330
	1000 µIU/ml	0.807
	3000 µIU/ml	1.933
	6000 µIU/ml	3.296

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 OD at zero binding, was 11 µIU/ml.

B. Specificity

The Cross-reactivity of LH, FSH, hCG, hPL, TSH and hGH was determined by addition of each analyte to serum samples containing respectively 116 µIU/ml and 2539 µIU/ml of PRL. The apparent PRL concentration was measured. As shown below, the cross-reactions with LH, FSH, hCG, hPL, TSH and hGH are insignificant

Added analyte to a low PRL value serum		Observed PRL value µIU/ml	Added analyte to a high PRL value serum		Observed PRL value µIU/ml
-		116	-		2539
LH	200 mIU/ml	120	LH	200 mIU/ml	2764
FSH	100 mIU/ml	114	FSH	100 mIU/ml	2497
hCG	100 IU/ml	115	hCG	100 IU/ml	2677
hPL	10000 ng/ml	123	hPL	10000 ng/ml	2517
TSH	200 µIU/ml	132	TSH	200 µIU/ml	2648
hGH	2000 ng/ml	157	hGH	2000 ng/ml	2791

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	<X> ± SD (µIU/ml)	CV (%)	Serum	N	<X> ± SD (µIU/ml)	CV (%)
A	20	505 ± 31	6.1	A	20	185 ± 13	6.9
B	20	1564 ± 73	4.7	B	20	1506 ± 90	6.0

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added PRL (µIU/ml)	Recovered PRL (µIU/ml)	Recovery (%)
Serum	50	53	106
	106	104	98
	331	321	97
	1069	1156	108
	2778	2534	91
Plasma	50	47	94
	106	101	96
	331	358	108
	1069	1243	116
	2778	2724	98

DILUTION TEST

Sample	Dilution	Theoretical Concent. (µIU/ml)	Measured Concent. (µIU/ml)
Serum	1/4	-	3491
	1/8	1745	1718
	1/16	873	901
	1/32	436	457
	1/64	218	240
	1/128	109	133
	1/256	55	55
	1/512	27	24
Plasma	1/1	-	951
	1/2	476	505
	1/4	238	290
	1/8	119	115
	1/16	59	57

Samples were diluted with zero calibrator.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 40 minutes after the calibrators have been added to the coated wells.

TIME DELAY					
	T0	10 min	20 min	30 min	40 min
S1	1078	965	965	1025	950
S2	437	428	408	431	447
S3	115	81	104	101	84

F. Hook effect

A sample spiked with PRL up to 420000 μ IU/ml gives higher OD's than the last calibrator point.

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 duplo results of the samples should rely on Good Laboratory Practices
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

XV. REFERENCE INTERVALS

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

Healthy Adults	N	Mean (μ IU/ml)	Range (μ IU/ml)
Males	143	66	8 - 201
Pre-menopausal women	121	103	10 - 526
Post-menopausal women	50	172	22 - 811

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

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 all reagents, Stop Solution contains H₂SO₄, the chromogen contains TMB in Dimethylformamide, Substrate buffer contains H₂O₂. In case of contact, wash thoroughly with water.

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

1. ARCHER D.F. (1977).
Current concepts of prolactin physiology in normal and abnormal conditions.
Fertil Steril 28:125.
2. LAUFER N., BOTERO-RUIZ W., DE CHEMEY A.H., et al. (1984).
Gonadotropin and prolactin levels in follicular fluid of human ova successfully fertilized in vitro.
J. Clin. Endocrinol. Metab. 58:430.

3. LEONG D.A., FRAWLEY L.S., NEIL J.D. (1983).
Neuroendocrine control of prolactin secretion.
An. Rev. of Physiol. 45:109.
4. SEPPALA M. (1978).
Prolactin and female reproduction.
An. Clin. Res. 10:164.
5. TAYLOR T.J., TROUSON A, BESANKO M., BURGER H.G., STOCKDALE J. (1986).
Plasma progesterone and prolactin changes in superovulated women before, during and immediately after laparoscopy for in vitro fertilisation and their relation to pregnancy
Fertil. Steril 45:680.
6. TYSON J.E. (1980)
Changing role of placental lactogen and prolactin in human gestation.
Clin. Obstet.Gynecol 23:737.
7. KAMEL M.A et al (1994)
Comparison between prolactin, gonadotropins and steroid hormones in serum and follicular fluid after stimulation with gonadotrophin-releasing hormone agonist and human menopausal gonadotrophin for an in-vitro fertilization program.
Hum. Reprod. 9(10):1803-6.
8. PATEL D.D. et al (1994).
Plasma prolactin in patients with colorectal cancer. Value in follow-up and as a prognosticator
Cancer 73(3):570-74.
9. HATTORI et al (1994).
Effects of anti-prolactin autoantibodies on serum prolactin measurements.
Eur. J. Endocrinol. 130(5):434-7.

XVIII. SUMMARY OF THE PROTOCOL

	CALIBRATORS (μ l)	SAMPLE(S) CONTROLS (μ l)
Calibrators (0-5) Samples, Controls Anti-PRL-HRP conjugate	25 - 100	- 25 100
Incubate for 30 min at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 μ l of Wash Solution and aspirate.		
Revelation Solution	200	200
Incubate for 15 min at room temperature with continuous shaking at 700 rpm.		
Stop Solution	50	50
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm) and 490 nm (versus 630 or 650 nm)		

Bio-Line Catalogue Nr : BL-25-E	Version : 040702-BL	Revision nr : 031113/1
------------------------------------	------------------------	---------------------------