AB Diagnostic Systems

REF **DK.044.01.9** Σ **96**

CE

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia PRL
Enzyme immunoassay for the quantitative determination of prolactin (PRL) concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (breakable wells) including controls; partial use of the kit is possible; can be used for manual protocol.

I. INTENDED USE

The abia PRL kit is intended for the quantitative determination of Prolactin (PRL) concentration in human serum by a microplate immunoenzymometric assay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Prolactin (PRL) is a polypeptide hormone synthesized by the lactotropic cells of the anterior pituitary gland. Structurally, it is similar to two other polypeptide hormones namely, growth hormone and placental lactogen. PRL is a polypeptide containing 199 amino acids, while growth hormone and placental lactogen each have 191 amino acids. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 22500 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor. Women normally have slightly higher basal prolactin levels than men; apparently there is an estrogen-related rise at puberty and corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactaition. Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10 and 20 times of normal values, declining to non-pregnant levels by 3-4 weeks post-partum. Breast feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. Prolactinomas are pituitary tumours secreting prolactin, found most frequently in females. High prolactin levels are commonly associated with galactorrhea and amenorrhea. In men some degree of impotency accompanied by a low testosterone level occurs, followed by azospermia. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanisms. Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account.

III. PRINCIPLE OF THE TEST

The abia PRL is a one-step immunoassay, based on principle of "sandwich" method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact PRL molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the PRL molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with washing solution to remove unbound labeled antibodies. A solution of TMB-Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The concentration of PRL is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT abia PRL

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-PRL-coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to PRL. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Monoclonal anti-PRL antibodies conjugated to horseradish peroxidase. Transparent or opalescent pink color liquid. Preserving agent: 0.05% ProClin 300, 0.004% gentamycin sulfate, 0.1% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4 Calibrator 5	Six vials containing PRL in protein-based buffer. Calibrators were calibrated using a WHO 4th IS 83/573 Standart. The prolactin concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquids, pale yellow. Preserving agent: 0.05% ProClin 300, 0.004% gentamycin sulfate, 0.1% phenol. Store at 2-8 °C until expiration date in tightly sealed vials.	5 vials 0.5 ml. Calibrator 0 – 2.0 ml
Control Serum	Protein based buffer with a defined quantity of Prolactin. The prolactin concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquid, pale yellow. Preserving agent: 0.05% ProClin 300, 0.004% gentamycin sulfate, 0.1% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 0.5 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric buffer solution, containing H_2O_2 (0.01%). Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
Protective films for EIA plates		2
Disposable tips		16
Disposable plastic dishes for liquid reagents		2
Polyethylene bag with a Zip-Lock		1

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24°C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and **kit cannot be used**.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with distilled or deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Do not let the wells dry once the assay has been started.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Control Serum and Calibrators has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).

- Certain reagents contain biological material of animal origin.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Calibrators 0-5, Control Serum contain ProClin 300.

H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm filter.
- Open type automated analyzer with 450 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Serum only may be used. Human serum or plasma collected in sodium heparin, lithium heparin, or potassium EDTA may be used in the assay. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Separated serum should be stored for no more 2 days at 2 to 8 °C. If samples are to be stored for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia and which were preserved by sodium azide must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-PRL-coated microtiter wells.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Calibrators 0-5;**
- **Control Serum;**
- **Conjugate;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution.** Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. To the wells add 25 µl of Calibrators and Control Serum in duplicates. Leave two wells for OD control of TMB-Substrate (blank).

2. To the rest of the wells, add 25 µl of samples in duplicates. Pipetting of samples should not extend beyond ten (10) minutes.

3. Add 100 µl of Conjugate to all wells except for the wells for OD control of TMB-Substrate (blank).

4. Swirl the microplate gently for 30 seconds after adding of samples and Conjugate to mix, cover the strips with a protective film and incubate for 90 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the wells 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbance paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

6. Pipette 100 µl of TMB-Substrate into each well.

7. Incubate for 15-20 minutes at room temperature in a dark place.

8. Stop the reaction by adding 150 µl of Stopping Reagent to the wells, shake the strips for 5-10 seconds and read the results. The time between stopping the reaction and measuring OD should not exceed 20 min.

9. Read the absorbance on the microplate reader at 450 nm.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of Conjugate + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and Conjugate must have an OD higher than 0.500.

10. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider “dead” volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. CALCULATION OF RESULTS

1. Calculate the mean absorbance value of each calibrator duplicate.
2. Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
3. Calculate the mean absorbance values for each specimen.
4. Read the values of the unknowns directly off the calibration curve, if immunoassay software is being used, a 4-parameter curve is recommended.
5. If a sample reads more than value of Calibrator 5 then dilute it with Calibrator 0. The result obtained should be multiplied by the dilution factor.

Typical tabulated data:

Calibrator	OD1	OD2	Mean OD-blank	Value (mIU/l)
1	0.138	0.143	0.098	125
2	0.426	0.428	0.385	500
3	0.782	0.79	0.744	1000
4	1.772	1.735	1.711	2250
5	2.812	2.888	2.808	4500
Unknown	0.402	0.407	0.362	494.4

This data is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Blank OD**: The absorbance value should not be more than 0.1 at 450 nm.
2. The absorbance (OD) of **Calibrator 5** should not be less than 1.3 at 450 nm.
3. Calculated Value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia PRL

1. Assay Dynamic Range

The range of the assay is between 0-4500 mIU/l.

2. Analytical sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 25 replicate analyses) plus 2 SD.

Therefore, the sensitivity of the abia PRL kit is 10 mIU/l.

Prolactin (LoD) sensitivity limit has been tested using WHO 4rd IS 83/573 according to CLSI EP17-A2 protocol. The LoD of the kit abia PRL consisted 22 mIU/l.

3. Limit of quantitation

The Limit of quantitation (LoQ) was defined using NCCLS EP 17 protocol. LoQ of the kit abia PRL is 30.0 mIU/l.

4. Specificity (cross reactivity)

The following substances were tested for cross reactivity of the assay:

Substance	Concentration tested, ng/ml	% Cross-reactivity	
		Prolactin High sample 50 ng/ml	Prolactin Low sample 10 ng/ml
FSH	2000	0.0987	0.0003
hCG	2000	0.0077	0.0000
LH	2000	0.0090	0.0002
TSH	2000	0.0010	0.0000

5. Interferences

Samples, containing up to 60 mg/dl bilirubin, lipemic samples containing up to 1500 mg/dl intralipid and haemolysed samples containing up to 2000 mg/dl hemoglobin do not affect the results.

6. Single-site precision

Repeatability, between-run precision, between-day precision of 2 lots were evaluated by testing 5 serum pools 2 times each during 20 days. The variation coefficient did not exceed 8%. Total precision was found less than 15%.

Serum Pool #	Mean mIU/l	Repeatability		Precision between Run (lots)		Precision between day		Total precision	
		SD	CV,%	SD	CV, %	SD	CV, %	SD	CV, %
1	1022.5	27.0	2.6	63.3	6.2	0.00	-	68.8	6.7
2	773.5	31.6	4.1	40.5	5.2	0.00	-	51.3	6.6
3	588.2	27.6	4.7	33.7	5.7	0.00	-	43.5	7.4
4	198.1	8.6	4.4	11.3	5.7	0.00	-	15.1	7.6
5	131.7	5.7	4.4	7.6	5.8	8.8	6.7	12.96	9.8

7. Multisite precision

Repeatability, between-run precision, within-laboratory precision and between site precision of one reagent lot were evaluated by testing 5 serum pools 5 times each during 5 days in three sites. The variation coefficient did not exceed 8%. Total precision was found less than 15%.

Sample	Mean mIU/l	Repeatability		Between-Run		Within-Laboratory		Between-Site		Reproducibility	
		SD	CV.%	SD	CV.%	SD	CV.%	SD	CV.%	SD	CV/%
1	1018.6	27.7	2.7%	39.2	3.9	48.0	4.7%	64.5	6.3	80.4	7.9%
2	592.3	19.4	3.3%	26.6	4.5	32.9	5.6%	9.2	1.6	34.2	5.8%
3	193.2	10.7	5.5%	7.7	4.0	13.2	6.8%	3.1	1.6	13.6	7.0%
4	156.0	9.6	6.1%	7.9	5.1	12.4	8.0%	4.2	2.7	13.1	8.4%

8. Recovery

Samples were prepared by adding defined amounts of PRL to patient serum sample. The results (in mIU/l) are tabulated below:

Sample	**Measured concentration, mIU/l**	**Expected concentration, mIU/l**	**Recovery, %**
Serum sample	713.8	-	-
Calibrator 2	515	-	-
Serum sample + Calibrator 2	612.7	614.4	99.7%

9. Linearity

The high Prolactin concentration serum sample (H) was diluted with Calibrator 0 which was used as the low-concentration sample (L). The results (in mIU/l) are tabulated below:

Dilution	Assigned value (mIU/l)	Measured Conc., mIU/l	Recovered, %
H	4600	4512	98.1
0.1L+0.9H	4140	4097	99.0
0.2L+0.8H	3680	3792	103.1
0.3L+0.7H	3220	3424	106.3
0.4L+0.6H	2760	2655	96.2
0.5L+0.5H	2300	2303	100.1
0.6L+0.4H	1840	1912	103.9
0.7L+0.3H	1380	1437	104.1
0.8L+0.2H	920	829	90.1
0.9L+0.1H	460	472	102.6
0.95L+0.05H	230	219	95.2
0.98L+0.02H	92	101	109.7

10. Expected normal Value

Group	Value, mIU/l
Male	73-572
Female	66-769

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Unit Conversion Calculator: ng/ml x 21.2 = mIU/l; mIU/l x 0.047 = ng/ml.

11. Accuracy

The abia PRL test system was compared with a with a commercially available CE marked kits as a reference test. The total number of specimens was 84. The values ranged from 153.2 to 3270.56 mIU/l. The least square regression equation and the correlation coefficient were computed for abia PRL in comparison with the reference method. The least square regression analysis was **y=0.9187(x) -28.8** with correlation coefficient 0.95.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the direct determination of PRL in human serum. The kit is not calibrated for the determination of PRL in saliva, plasma or other specimens of human or animal origin.
2. Any improper handing of samples or modification of this test might influence the results.
3. Only Calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

4. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient.

5. Pregnancy, estrogen treatment, renal disease, and hypothyroidism may affect prolactin levels.

6. Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

7. Patients specimens with abnormally high prolactin levels can lead a hook effect, that is, paradoxical low absorbance results. If this is suspected, dilute the specimen 1/100 with 0 Calibrator, reassay and multiply the result by 100. No hook effect was observed in this test until 100 000 mIU/l.

8. Not intended for newborn screening.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging.** Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in table 2.
- Transportation should be done by covered transport at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 2

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-20 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial.		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	Anti-PRL-coated microtiter wells	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock plastic bag. Do not remove desiccant.	Until the kit expiration date.
	Washing Solution, Stopping Reagent	Close the vials tightly with screw caps and stored them in the manufacturer's package.	Until the kit expiration date.
	Calibrators №0-5, Control Serum, Conjugate, TMB-Substrate	Close the vials tightly with screw caps and stored them in the manufacturer's package.	For two months.

XV. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer if you have any questions.

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XVII. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)	+8°C +2°C	Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD	Σ	Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Annex

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates two wells for TMB control (blank)
2	**Add**	100 µl of Conjugate into all wells, except for the wells for OD control of TMB-Substrate (blank)
3	**Mix**	30 seconds
4	**Incubate**	90 min, at 20-25 °C
5	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
6	**Add**	100 µl of TMB-Substrate into all wells
7	**Incubate**	15-20 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	5-10 seconds
10	**Read the optical density**	450 nm

ng/ml x 21.2 = mIU/l; mIU/l x 0.047 = ng/ml.