Monobind Inc.
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AccuLite
CLIA Microwells

Prolactin Hormone Sequential (PRLs) Test System

Product Code: 4475-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Prolactin Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prolactin hormone (PRL), secreted from the lactotrophs of the anterior pituitary, is a protein consisting of a single polypeptide chain containing approximately 200 amino acids. The primary biological action of the hormone is on the mammary gland where it is involved in the growth of the gland and in the induction and maintenance of milk production. There is evidence to suggest that prolactin may be involved in steroidogenesis in the gonad, acting synergistically with luteinizing hormone (LH). High levels of prolactin appear to inhibit steroidogenesis as well as inhibiting LH and follicle stimulating hormone (FSH) synthesis at the pituitary gland.[1,2]

The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent monitoring the effectiveness of the treatment has been well established.[3,4]

In this method, PRL calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for PRL) is added and the reactants mixed. Reaction between the PRL antibodies and native PRL forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. A tracer labeled monoclonal antibody specific to PRL (different epitope from the biotinylated antibody) is added to the wells. The tracer labeled antibody binds to the PRL already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess tracer is washed off via a wash step. Light is generated by the addition of a substrate. The intensity of the light intensity is directly proportional to the concentration of the PRL in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-prolactin antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(Prl)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(Prl)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(Prl)}$ = Native Antigen (Variable Quantity)
$Ag_{(Prl)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(Prl)} - {}^{Btn}Ab_{(m)} + \underline{\text{Streptavidin}}_{CW} \Rightarrow \underline{\text{Immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{CW}$ = Streptavidin immobilized on well
$\underline{\text{Immobilized complex (IC)}}$ = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce light measurable with the use of a microplate luminometer. The light produced on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}Prl)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}Prl)} - IC$$

${}^{Enz}Ab_{(x\text{-}Prl)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}Prl)}$ - IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. PRL-seq Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for PRL antigen in human serum at levels of 0(**A**), 10(**B**), 25(**C**), 50(**D**), 100(**E**) and 250(**F**) ng/ml*. Store at 2-8°C. A preservative has been added.
***Note**: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd, IS (84/500).

B. PRLs Biotin Reagent – 13ml/vial - Icon ∇
One (1) vial contains biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. PRLs Tracer Reagent – 13ml/vial - Icon
One (1) vial containing enzyme (HRP) labeled antibody, in buffer, dye, and preservative. Store at 2-8°C.

D. Light Reaction Wells – 96 wells – Icon ⇓
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reagent A – 7ml/vial - Icon C[A]
One (1) vial contains luminol in buffer. Store at 2-8°C.

G. Signal Reagent B – 7ml/vial - Icon C[B]
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Test tube(s) for mixing substrates A&B.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution –** Store at 2 – 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of PRLs Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of PRLs Tracer Reagent solution to all wells.
DO NOT SHAKE THE PLATE AFTER TRACER ADDITION
9. Incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
12. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
13. Incubate at room temperature in the dark for five (5) minutes.
14. Read the relative light units in each well, for minimum 0.5 – 1.0 seconds, using a microplate luminometer. **The results should be read within thirty (30) minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of prolactin (PRL) concentration in unknown specimens.
1. Record the RLUs (Relative Light Unit) obtained from the printout of the luminometer as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding PRL concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hPRL for an unknown, locate the average RLUs of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (16513) of the patient intersects the calibration curve at (24.8ngml) PRL concentration (See Figure 1).

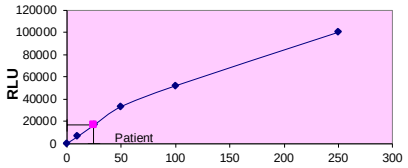
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (ng/ml)
Cal A	A1	48	48	0
	B1	47		
Cal B	C1	6133	6250	10
	D1	6367		
Cal C	E1	16557	24603	25
	F1	16549		
Cal D	G1	33320	53344	50
	H1	32996		
Cal E	A2	52253	77335	100
	B2	51451		
Cal F	C2	100792	100000	250
	D2	99208		
Ctrl 1	E2	7390	7268	**11.5**
	F2	7146		
Patient	G2	16522	16513	**24.8**
	H2	16774		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Patient specimens with abnormally high prolactin levels will not cause a hook effect, due to the assay design (sequential method). For specimens with values greater than 250, dilute the specimen 1/50 with 0 calibrator; and re-assay (multiply the result by 50).
8. Patients receiving preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.
9. Pregnancy, lactation, and the administration of oral contraceptives can cause an increase in the level of Prolactin.
10. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion.[5,6,7]
11. Since Prolactin hormone concentration is dependent upon diverse factors other than pituitary homeostasis, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the PRLs AccuLite® CLIA Test System. The expected values (95% confidence intervals) are presented in Table 1.

TABLE I
Expected Values for the PRLs AccuLite® CLIA (in ng/ml)

	Women
Adult (Number = 70)	1.2 -- 19.5
Postmenopausal (Number = 10)	1.5 -- 18.5
	Men
Adult (Number = 50)	1.8 -- 17.0

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the PRLs AccuLite® CLIA procedure were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	24	8.4	0.43	5.1%
Level 2	24	20.4	0.95	4.7%
Level 3	24	90.6	3.44	3.8%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	8.7	0.56	6.4%
Level 2	10	20.1	1.02	5.1%
Level 3	10	93.5	4.21	4.5%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

This procedure has a sensitivity of 0.02 ng. This is equivalent to a sample containing 0.8 ng/ml PRL concentration. The sensitivity was ascertained by determining the variability of the '0ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The PRLs AccuLite® CLIA procedure was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 86. The least square regression equation and the correlation coefficient were computed for the PRL CLIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	19.0	y = 1.63 + 1.01(x)	0.973
Reference	17.3		

Only slight amounts of bias between the PRLs AccuLite® CLIA procedure and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross reactivity of the prolactin hormone method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of prolactin hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Prolactin Hormone (PRL)	1.0000	--
Luteinizing Hormone (LH)	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml
Growth Hormone (GH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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MP4475 **Product Code: *4475-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 (13ml)	2 (13ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)

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IVD 2° 8° CE
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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity