



---

## PROLACTIN (PRL) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1103-2

### **INTENDED USE**

The Autobio prolactin (PRL) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of PRL concentration in human serum.

### **INTRODUCTION**

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 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 a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10 and 20 times 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. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism. 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. Prolactin concentrations may also be increased by drugs such as chlorpromazine and reserpine, and may be lowered by bromocryptine and L-dopa.

### **PRINCIPLE OF THE TEST**

The PRL CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The PRL molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of PRL present in the sample.

### **MATERIALS PROVIDED**

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to prolactin (anti-PRL MAb) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-PRL MAb in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 50, 150, 500, 1000 and 3000 $\mu$ IU/ml PRL in Stabilizing Buffer. (5 vials, lyophilized)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)
6. PBS-T powder: PBS-Tween (1bag, 5g)

### **MATERIALS NOT PROVIDED**

The following materials are required but not provided in the kit.

1. Precision pipettes and tips, 0.025ml, 0.1ml, 1.0ml,
2. Distilled water.
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Luminometer

---

**STORAGE OF TEST KIT AND INSTRUMENTATION**

1. Unopened test kits should be stored at 2~8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

**SPECIMEN COLLECTION AND PREPARATION**

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2~8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

**PRECAUTIONS AND WARNINGS**

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18~25°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2~8°C. Reconstituted standards should be used within 14 days and be frozen at -20°C for long term storage.
3. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.

**IMPORTANT NOTES**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Dispense 25µl of Reference Standards, specimens, and controls into appropriate wells.
2. Dispense 100µl of Enzyme Conjugate Reagent to each well. Mix gently for 30 seconds.
3. Incubate for 60 minutes at 37°C.
4. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300µl.
5. Dispense 50µl substrate A and 50µl substrate B reagent into each well. Gently mix for 10 seconds.
6. Incubate at room temperature in the dark for 5 minutes without shaking and read the RLU values with a Luminometer.

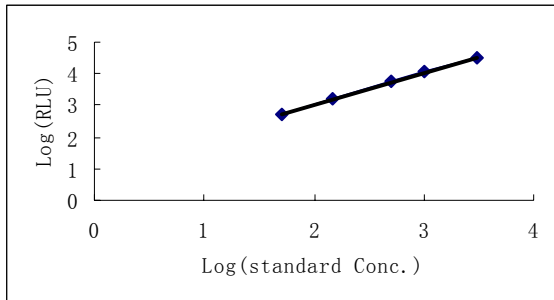
**CALCULATION OF RESULTS**

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Plot the  $\log_{10}$ RLU for each reference standard against the common logarithm of corresponding concentration of PRL in mIU/ml on logarithmic graph paper, with RLU values on the Y-axis and and concentration on the X- axis.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

**EXAMPLE OF STANDARD CURVE**

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

PRL ( $\mu$ IU/ml)	RLU
50	526.924
150	1532.12
500	5799.25
1000	11418.2
3000	33270.9



**EXPECTED VALUES**

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected out-patient clinical laboratory samples:

	Normal Range ( $\mu$ IU/ml)
Male	42.5 ~ 414.0
Female	51.0 ~ 580.0

**PERFORMANCE**

**A. Sensitivity**

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio PRL CLIA kit is not higher than 25 $\mu$ IU/ml.

**B. Specificity**

No interference was detected with the performance of Autobio PRL CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
FSH	500mIU/ml
hCG	100,000mIU/ml
HGH	100ng/ml
HPL	100ng/ml
LH	500mIU/ml
TSH	500 $\mu$ IU/ml

**C. Precision**

**a. Intra-assay Precision**

Intra-Assay Precision was determined by assaying 10 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	10	267.32	5.64	2.11
High titer	10	673.79	17.01	2.52

**b. Inter-assay Precision**

Inter-assay Precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	264.21	7.32	2.77
High titer	10	691.03	19.31	2.79

**D. High Dose Hook Effect**

In this PRL assay, patient samples spiked to PRL levels up to 43,000µIU/ml do not demonstrate a paradoxical decrease in the RLU's (high dose hook effect).

**E. Accuracy**

For 180 specimens in the range of 25µIU/ml I to 3000µIU/ml, the relationship between the Autobio PRL CLIA and Beckman Access 2® assay is described by the equation below:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation coefficient
Beckman access 2®	180	$y = 0.9432x + 0.8372$	0.9712

**LIMITATIONS**



1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

**QUALITY CONTROL**

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

**SYMBOLS**


	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION

	CATALOGUE NUMBER
	CONSULT INSTRUCTIONS FOR USE

**REFERENCES**

1. Uotila M., Ruuslahti E. and Engvall E. J. Immunol. Methods 1981; 42: 11-15
2. Shome B. and Parlow A.F. J. Clin. Endocrinol. Metab. 1977; 45: 1112-1115
3. Cowden E.A., Ratcliffe W.A., Beastall G.H. and Ratcliffe J.G. Annals Clin. Biochem 1979; 16: 113-121
4. Frantz A.G. N. Engl. J. Med. 1978; 298: 201-207
5. Jacobs L., Snyder P., Wilber J., Utiger R. and Daughaday W. J. Clin. Endocrin. 1978; 33: 99

**For order and inquires, please contact**

	<p>AUTOBIO DIAGNOSTICS CO., LTD.                  ADD: No.87 Jingbei Yi Road, National Eco &amp; Tech Development Area,                  Zhengzhou , China 450016                  Tel: +86-371-67985313 Fax: +86-371-67985804                  Web: www.autobio.com.cn</p>
---	---