

## **Prolactin ELISA Kit Protocol**

(Cat. No.:EK-310-37)

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## **INTENDED USE**

For the quantitative determination of prolactin concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

## **LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

## TABLE OF CONTENT

Introduction and Protocol Overview	4
Principle of the Test	5
List of Components	5
Specimen Collection and Preparation	6
Storage	6
Reagent Preparation	6
Assay Procedure	6
Calculation of Results	7
Example of Standard Curve	8
Expected Values and Sensitivity	9
References	9

### INTRODUCTION AND PROTOCOL OVERVIEW

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and woman. 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 to 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 estrogen, 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 bromocriptine and L-dopa.

**CAUTION:** Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

### PRINCIPLE OF THE TEST

The Prolactin Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

### LIST OF COMPONENTS

#### Materials Provided with the Kit:

- Antibody coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- Prolactin reference standards, containing 0, 5, 15, 50, 100 and 200 ng/ml (WHO, 1st IRP 75/504), lyophilized.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

#### Materials required but not provided:

- Precision pipettes: 50  $\mu$ l, 100  $\mu$ l and 1.0 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of

10 nm or less and an optical density range of 0-2 OD or greater.

- Graph paper.

### **SPECIMEN COLLECTION AND PREPARATION**

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

### **STORAGE**

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. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. **DO NOT FREEZE**

### **REAGENT PREPARATION**

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

### **ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Gently mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into sink.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to re-

move all residual water droplets.

9. Dispense 100  $\mu$ l TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100  $\mu$ l of Stop Solution to each well.
12. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
13. Read the optical density at 450nm with a microtiter plate reader **within 15 minutes.**

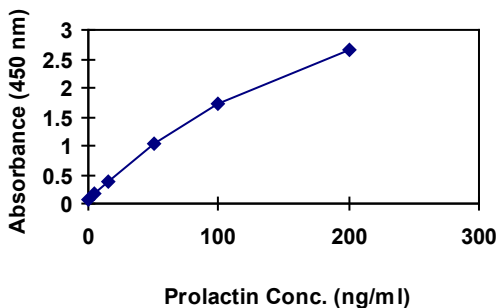
### CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of prolactin in ng/ml from the standard curve.

## EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y-axis against Prolactin concentrations shown in the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and patient data in each experiment.

Prolactin (ng/ml)	Absorbance (450 nm)
0	0.052
5	0.166
15	0.383
50	1.047
100	1.737
200	2.644





## EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on patient population. The minimal detectable concentration of human prolactin by this assay is estimated to be 2 ng/ml. The information provided below is cited from Reference #6.

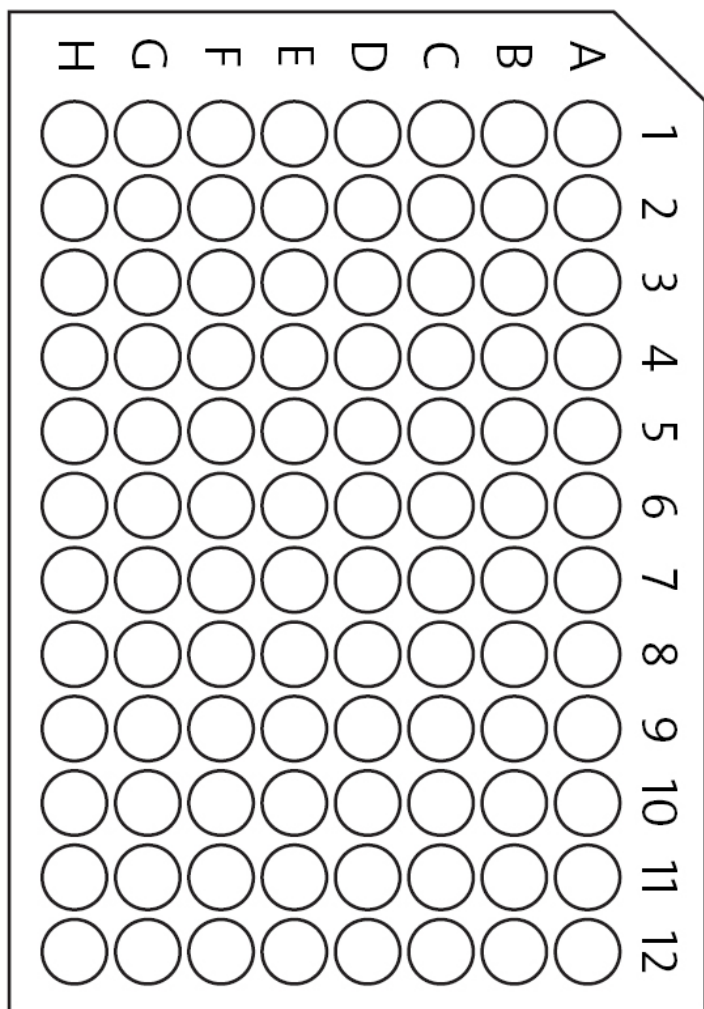
Adult	ng/ml
Male	3.0-14.7
Female	
Pregnancy,	3.8-23.2
Third trimester:	95-473

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## REFERENCES

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# ASSAY DIAGRAM



## **NOTES**