# Human Prolactin ELISA Kit

For the quantitative determination of human Prolactin concentrations in serum

Catalogue Number: EL10014

*96 tests*

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

*ANOGEN*

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

S7.5 (03) Prolactin

## TABLE OF CONTENTS


| | Page |
|---|---|
| INTENDED USE | 2 |
| INTRODUCTION | 2 |
| PRINCIPLE OF THE ASSAY | 2 |
| REAGENTS PROVIDED | 3 |
| MATERIALS REQUIRED BUT NOT SUPPLIED | 4 |
| PRECAUTIONS | 4 |
| SAMPLE PREPARATION | 5 |
| .............Collection, Handling, and Storage | 5 |
| PREPARATION OF REAGENTS | 5 |
| ASSAY PROCEDURE | 6 |
| CALCULATION OF RESULTS | 7 |
| TYPICAL DATA | 7 |
| .............Example | 7 |
| PERFORMANCE CHARACTERICS | 8 |
| .............Sensitivity | 8 |
| .............Specificity | 8 |
| .............Calibration | 8 |
| .............Hook effect | 8 |
| .............Expected Normal Values | 8 |
| CITATIONS | 9 |

## INTENDED USE

This Human Prolactin ELISA Kit is to be used for the *in vitro* quantitative determination of human Prolactin concentrations in serum. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

## 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. The release and synthesis of Prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Hormone and Prolactin Inhibiting Hormone.

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 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. Prolactin concentrations may also be increased by drugs such as chloropromazine and reserpine and may be lowered by bromocriptine and L-dopa.

## PRINCIPLE OF THE ASSAY

This Prolactin enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for Prolactin. Standards or samples are then added to the microtiter plate wells and Prolactin if present, will bind to the antibody pre-coated on the wells. In order to quantitate the amount of Prolactin present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody, specific for Prolactin are added to each well to “sandwich” the Prolactin immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain Prolactin and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of Prolactin in the sample, this Human Prolactin ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the

same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus Prolactin concentration (ng/mL). The concentration of Prolactin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1. **MICROTITER PLATE** (Part EL14-1) ***96 tests*** ***96 wells***
   Pre-coated with anti-human Prolactin monoclonal antibody.
2. **CONJUGATE** (Part EL14-2) ***12 mL***
   Anti-human Prolactin monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. *Ready-to-use.*
3. **STANDARD - 200 ng/mL** (Part EL14-3) ***1 vial***
   Lyophilized human Prolactin in a buffered protein base with preservative that will contain 200 ng/mL after reconstitution.
4. **STANDARD - 100 ng/mL** (Part EL14-4) ***1 vial***
   Lyophilized human Prolactin in a buffered protein base with preservative that will contain 100 ng/mL after reconstitution.
5. **STANDARD - 50 ng/mL** (Part EL14-5) ***1 vial***
   Lyophilized human Prolactin in a buffered protein base with preservative that will contain 50 ng/mL after reconstitution.
6. **STANDARD - 25 ng/mL** (Part EL14-6) ***1 vial***
   Lyophilized human Prolactin in a buffered protein base with preservative that will contain 25 ng/mL after reconstitution.
7. **STANDARD - 5 ng/mL** (Part EL14-7) ***1 vial***
   Lyophilized human Prolactin in a buffered protein base with preservative that will contain 5 ng/mL after reconstitution.
8. **STANDARD - 0 ng/mL** (Part EL14-8) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0 ng/mL after reconstitution.
9. **SUBSTRATE A** (Part EL14-9) ***10 mL***
   Buffered solution with $H_20_2$.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid ($H_2SO_4$). Caution: Caustic Material!

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 µL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator (37±2°C)
8. Microtiter plate reader (450 nm±2 nm)
9. Automatic microtiter plate washer or squirt bottle
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.

**PRECAUTIONS**

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

## SAMPLE PREPARATION

### COLLECTION, HANDLING, AND STORAGE

**Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

## PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Prolactin Standards:** Reconstitute each Prolactin Standard vial with **0.6 mL** of distilled or deionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. The Prolactin standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

| Wells Used | Substrate A (mL) | Substrate B (mL) | Substrate Solution (mL) |
|---|---|---|---|
| 16 wells | 1.5 | 1.5 | 3.0 |
| 32 wells | 3.0 | 3.0 | 6.0 |
| 48 wells | 4.0 | 4.0 | 8.0 |
| 64 wells | 5.0 | 5.0 | 10.0 |
| 80-wells | 6.0 | 6.0 | 12.0 |
| 96 wells | 7.0 | 7.0 | 14.0 |

## ASSAY PROCEDURE

1. Prepare all Prolactin Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. First, secure the desired number of coated wells in the holder, then add 50 μL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate.

3. Add 100 μL of Conjugate to each well. <u>COMPLETE MIXING IN THIS STEP IS IMPORTANT</u>. Cover and incubate for **1 hour at 37°C**.

4. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).

5. Wash the Microtiter Plate using one of the specified methods indicated below:

   <u>Manual Washing</u>: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   <u>Automated Washing</u>: Aspirate all wells, then wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to apirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

6. Add 100 μL Substrate Solution to each well. Cover and incubate for **15 minutes at 37°C**.

7. Add 100 μL of Stop Solution to each well. Mix well.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

## CALCULATION OF RESULTS

This standard curve is used to determine the amount of Prolactin in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding Prolactin concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 ng/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of Prolactin in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding Prolactin concentration.

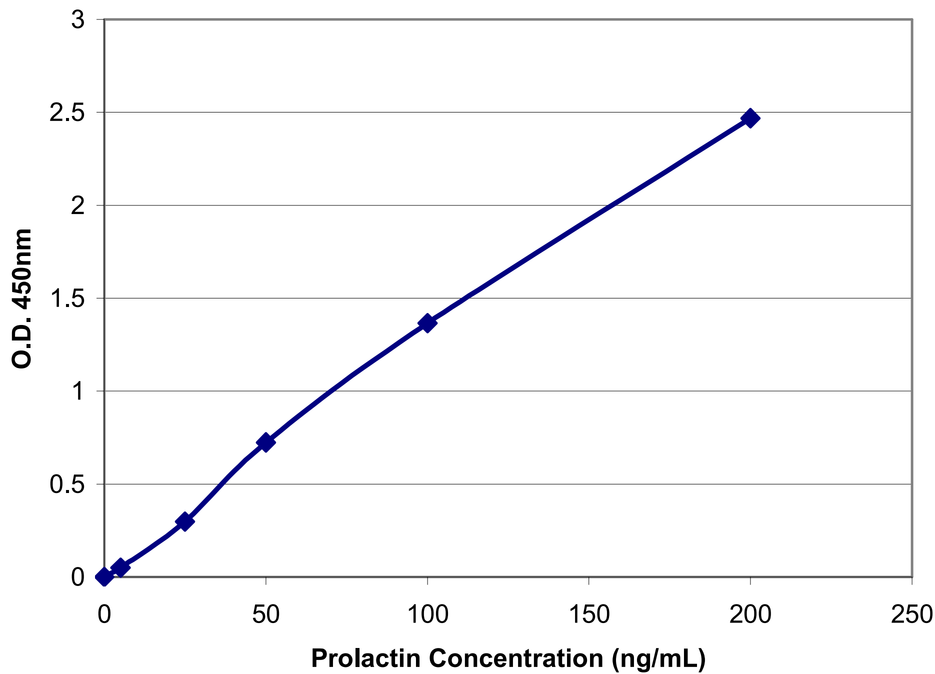
## TYPICAL DATA

Results of a typical standard run of Prolactin ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

### EXAMPLE

Results of a typical standard run are shown below:

| Standard (ng/mL) | O.D. (450 nm) | Mean | Zero Standard Subtracted |
|---|---|---|---|
| 0 | 0.030, 0.028 | 0.029 | 0 |
| 5 | 0.080, 0.079 | 0.080 | 0.051 |
| 25 | 0.324, 0.332 | 0.328 | 0.299 |
| 50 | 0.755, 0.750 | 0.753 | 0.724 |
| 100 | 1.383, 1.406 | 1.395 | 1.366 |
| 200 | 2.514, 2.480 | 2.497 | 2.468 |




## PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY**
   The minimal detectable concentration of human Prolactin by this assay is estimated to be 1.5 ng/mL.

2. **SPECIFICITY**
   This kit exhibits no detectable cross-reaction with human FSH, LH, and TSH, hCG and hGH.

3. **CALIBRATION**
   This immunoassay is calibrated against WHO, 3rd IS, 84/500.

4. **HOOK EFFECT**
   In this assay, no hook effect is observed up to 10,000 ng/mL.

5. **EXPECTED NORMAL VALUES**
   Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on a limited number of healthy adult blood specimens.

| | *MALE* | *FEMALE* |
|---|---|---|
| N | 90 | 120 |
| Mean (ng/mL) | 6.0 | 15.0 |
| Range (ng/mL) | 1.0-12.0 | 8.0-21.0 |

## CITATIONS

1. Tsinzerling N et al. Raised prolactin levels in myasthenia gravis: two case reports and a study of two patient populations. Acta Neurol Scand. 2006 Nov;114(5):346-9.

2. Q Xu et al. Isolation of tumour stem-like cells from benign tumours. Br J Cancer. Jul 21, 2009; 101(2): 303–311.