Human Prolactin
ELISA kit
Catalog # LF-EK60014 (1 kit)

*Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of Human Prolactin*

For research use only
Not for diagnostic or therapeutic procedures
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1. 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 postpartum. 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.

2. Principles of Method

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.

3. 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.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -20°C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.
### 6. Kit Contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Number</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin MICROTITER PLATE</td>
<td>1 (in aluminum foil bag with desiccant)</td>
<td></td>
</tr>
<tr>
<td>Conjugate</td>
<td>1</td>
<td>12 mL</td>
</tr>
<tr>
<td>STANDARD- 200 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>STANDARD- 100 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>STANDARD - 50 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>STANDARD - 25 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>STANDARD - 5 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>STANDARD - 0 ng/mL</td>
<td>1 vials (lyophilized)</td>
<td></td>
</tr>
<tr>
<td>SUBSTRATE A (H₂O₂)</td>
<td>1</td>
<td>10 ml</td>
</tr>
<tr>
<td>SUBSTRATE B (TMB)</td>
<td>1</td>
<td>10 ml</td>
</tr>
<tr>
<td>STOP SOLUTION</td>
<td>1</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

1. **Prolactin MICROTITER PLATE**
   - Pre-coated with anti-human Prolactin monoclonal antibody.

2. **Conjugate**
   - Anti-human Prolactin monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. Ready-to-use.

3. **STANDARD- 200 ng/mL**
   - Lyophilized human Prolactin in a buffered protein base with preservative that will contain 200 ng/mL after reconstitution.

4. **STANDARD- 100 ng/mL**
   - Lyophilized human Prolactin in a buffered protein base with preservative that will contain 100 ng/mL after reconstitution.

5. **STANDARD - 50 ng/mL**
   - Lyophilized human Prolactin in a buffered protein base with preservative that will contain 50 ng/mL after reconstitution.

6. **STANDARD - 25 ng/mL**
   - Lyophilized human Prolactin in a buffered protein base with preservative that will contain 25 ng/mL after reconstitution.

7. **STANDARD - 5 ng/mL**
   - Lyophilized human Prolactin in a buffered protein base with preservative that will contain 5 ng/mL after reconstitution.

8. **STANDARD - 0 ng/mL**
   - Lyophilized buffered protein base with preservative that will contain 0 ng/mL after reconstitution.
9 SUBSTRATE A
: Buffered solution with H₂O₂.

10 SUBSTRATE B
: Buffered solution with TMB.

11 STOP SOLUTION
: 2N Sulphuric Acid (H₂SO₄). Caution: Caustic Material!
  • Do not mix or interchange different reagents from various kit lots.

7. Materials Required But Not Provided

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 uL 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. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Disposable gloves.
12. Absorbent paper towels

8. 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 Wastes:** Autoclave for 60 minutes at 121°C.
   **Liquid Wastes:** 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 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 and flame.
12) If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

9. Preparation

1) Sample preparation

**COLLECTION, HANDLING AND STORAGE**

a) **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) followed by serum extraction.
   • 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.**

**2) Preparation of Reagent**

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 provided for correct amounts of Substrate Solution to prepare.

<table>
<thead>
<tr>
<th>Strips Used</th>
<th>Substrate A (mL)</th>
<th>Substrate B (mL)</th>
<th>Substrate Solution (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 strips (16 wells)</td>
<td>1.5</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>4 strips (32 wells)</td>
<td>3.0</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td>6 strips (48 wells)</td>
<td>4.0</td>
<td>4.0</td>
<td>8.0</td>
</tr>
<tr>
<td>8 strips (64 wells)</td>
<td>5.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td>10 strips (80 wells)</td>
<td>6.0</td>
<td>6.0</td>
<td>12.0</td>
</tr>
<tr>
<td>12 strips (96 wells)</td>
<td>7.0</td>
<td>7.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

**10. 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 ul of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate.

3) Add 100 ul of Conjugate to each well. **COMPLETE MIXING IN THIS STEP IS IMPORTANT.** 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:

**Manual Washing:** Remove the incubation mixture by aspirating the contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with deionized or distilled water, and 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 the plate onto absorbent papers 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 the frame.

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

6) Add 100 ul of Conjugate into each well. Cover and incubate for 30 minutes at 37°C

7) Add 100 ul Stop Solution to each well. Mix well.

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

11. Characteristics

1) 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.
2) 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:

<table>
<thead>
<tr>
<th>Standard (ng/ml)</th>
<th>O.D (450nm)</th>
<th>Mean</th>
<th>Zero Standard Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.030, 0.028</td>
<td>0.029</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.080, 0.079</td>
<td>0.080</td>
<td>0.051</td>
</tr>
<tr>
<td>25</td>
<td>0.324, 0.332</td>
<td>0.328</td>
<td>0.299</td>
</tr>
<tr>
<td>50</td>
<td>0.755, 0.750</td>
<td>0.753</td>
<td>0.724</td>
</tr>
<tr>
<td>100</td>
<td>1.383, 1.406</td>
<td>1.395</td>
<td>1.366</td>
</tr>
<tr>
<td>200</td>
<td>2.514, 2.480</td>
<td>2.497</td>
<td>2.468</td>
</tr>
</tbody>
</table>

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

4) SPECIFICITY
This kit exhibits no detectable cross-reaction with human FSH, LH, and TSH, hCG and hGH.
5) CALIBRATION
This immunoassay is calibrated against WHO, 3rd IS, 84/500.

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

7) 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.

<table>
<thead>
<tr>
<th></th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>6.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Range (ng/ml)</td>
<td>1.0-12.0</td>
<td>8.0-21.0</td>
</tr>
</tbody>
</table>
# 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
</table>
| High signal and background in all wells | • Insufficient washing  
• Too much AV-HRP  
• Incubation time too long  
• Development time too long | • Increase number of washes  
• Increase time of soaking between in wash  
• Check dilution, titration  
• Reduce incubation time  
• Decrease the incubation time before the stop solution is added |
| No signal | • Reagent added in incorrect order, or incorrectly prepared  
• Standard has gone bad (If there is a signal in the sample wells)  
• Assay was conducted from an incorrect starting point | • Review protocol  
• Check the condition of stored standard  
• Reagents allows to come to 20~30℃ before performing assay |
| Too much signal – whole plate turned uniformly blue | • Insufficient washing – unbound AV-HRP remaining  
• Too much AV-HRP  
• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP | • Increase number of washes carefully  
• Check dilution  
• Use fresh plate sealer and reagent reservoir for each step |
| Standard curve achieved but poor discrimination between point | • Plate not developed long enough  
• Improper calculation of standard curve dilution | • Increase substrate solution incubation time  
• Check dilution, make new standard curve |
| No signal when a signal is expected, but standard curve looks fine | • Sample matrix is masking detection | • More diluted sample recommended |
| Samples are reading too high, but standard curve is fine | • Samples contain protein levels above assay range | • Dilute samples and run again |
| Edge effect | • Uneven temperature around work surface | • Avoid incubating plate in areas where environmental conditions vary  
• Use plate sealer |
 Ordering Information

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Website: http://www.abfrontier.com