SAFETY CAUTIONS AND WARNINGS

PROCELUT (PRL) ELISA

INTENDED USE

For the quantitative determination of prolactin (PRL) in human serum by an enzyme immunnoassay. For in-vitro diagnostic use only.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for a successful and reliable performance. The test result may only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assaying the reliability of results.
3. When the use of water is specified for dilution or reconstituting, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kits, reagents and samples.
5. If reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper or delayed washing after termination of the enzymatic reaction, may result in OD values higher than expected and may account for high results when assay values for the controls do not reflect established confidence limits.
9. When reading the microplate, the presence of bubbles in the wells may affect the optical densities (ODs). Carefully remove any bubbles before proceeding to the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain unopened and unexposed to light. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond its expiration date printed on the label.
14. Reagents and samples must be regarded as hazardous and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are prepared for the direct determination of prolactin in human serum. The kit is not calibrated for the determination of prolactin in other biological fluids such as cerebrospinal fluid, plasma or other specimens of human or animal origin.
2. Test samples should be grossly hematicised, grossly icteric or improperly stored.
3. Any samples or control sera containing azide or thimerosal as a preservative may lead to high results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagents will lead to inaccurate results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of clinical symptoms in a patient with a history of psychiatric disease may lead to a false positive PRL level.
6. If dopamine is not available or absent the secretion of PRL is inhibited; a direct effect of inhibition of the secretion of PRL. Its main physiological action is not only to initiate but also to sustain lactation. The hypothalamus secretes dopamine, which makes use of two highly specific monocular antibodies: A monoclonal antibody specific for each amino acid transporter to the microplate and another monoclonal antibody specific for a different region of prolactin to conjugate to horse radish peroxidase (HRP). Proteins and all standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After washing the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microplate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of prolactin in the sample. A set of standards is used to plot a standard curve from which the amount of prolactin in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Prolactin (PRL) is a polypeptide hormone synthesized by the lactotrophic cells of the anterior pituitary gland. Structurally, it consists of 191 amino acids. There is approximately 100 µg of PRL in each 2.0 mL of plasma. There are several forms of prolactin, including the frequency of exposure to animals/products if any that is left over.

SAFETY PRECAUTIONS AND STORAGA

Approximately 0.2 mL of serum is required for duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and immediately refrigerate at 4°C or below. Store the samples in the cold for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for antibodies to HCV and Human Immunodeficiency Virus (HIV) and that it is free from infectious contamination. The reagents should be considered a potential biohazard and handled with the same precautions as any other infectious blood.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Avoid using sample containing other potentially interfering substances. Store at 4°C for up to 24 hours or at -10°C or lower. If the analyses are to be done at a later date. Consider all human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for antibodies to HCV and Human Immunodeficiency Virus (HIV) and that it is free from infectious contamination. The reagents should be considered a potential biohazard and handled with the same precautions as any other infectious blood.

REAGENTS PROVIDED

1. Mouse Anti-Prolactin Antibody-Coated Break-Apart Well Microlute — Ready To Use
   Contents: One 96-well (128x) monoclonal antibody-coated microwell plate with disposable water.
   Storage: Refrigerate at 2–8°C.

2. Mouse Anti-Prolactin Antibody-Horseradish Peroxidase (HRP) Conjugate — Requires Preparation
   Contents: Anti-Prolactin antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C.

3. Prolactin Calibrators — Ready To Use
   Contents: Six vials containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of prolactin.
   Storage: Refrigerate at 2–8°C.

4. Controls — Ready To Use
   Contents: Two vials containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C.

5. Wash Buffer Concentrate — Requires Preparation
   Contents: One bottle containing buffer with a non-ionic surfactant and a non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C.

6. Assay Buffer — Ready To Use
   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C.

7. TMB Substrate — Ready To Use
   Contents: One bottle containing tetramethylbenzidine and peroxidase in a non-DMF or DMSO containing buffer.
   Volume: 12 mL/bottle
   Storage: Refrigerate at 2–8°C.

8. Stopping Solution — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C.

9. Controls — Ready To Use
   Contents: Two vials containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C.

10. Controls — Ready To Use
    Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
    Volume: 3 mL/vial
    Storage: Refrigerate at 2–8°C.

11. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 1 mL/vial
     Storage: Refrigerate at 2–8°C.

12. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 0.3 mL/vial
     Storage: Refrigerate at 2–8°C.

13. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 0.1 mL/vial
     Storage: Refrigerate at 2–8°C.

14. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 0.03 mL/vial
     Storage: Refrigerate at 2–8°C.

15. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 0.01 mL/vial
     Storage: Refrigerate at 2–8°C.

16. Controls — Ready To Use
     Contents: One bottle containing prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of prolactin.
     Volume: 0.003 mL/vial
     Storage: Refrigerate at 2–8°C.
ASSAY PROCEDURE
Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the conjugate and wash buffer (refer to reagents provided section).
2. Remove the required number of well strips. Reveal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 45 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same time intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

TYPICAL CALIBRATOR CURVE

Performance Characteristics
Sensitivity
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DBC prolactin ELISA kit is 10 µIU/mL.

Specificity (Cross-Reactivity)
The specificity of the DBC prolactin ELISA kit was determined by comparing the apparent prolactin values of the following compounds:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Apparent PRL Value (µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG (WHO 75/537)</td>
<td>100–2500 IUL</td>
<td>Not Detected</td>
</tr>
<tr>
<td>FSH (WHO 75/375)</td>
<td>25–4000 IUL</td>
<td>Not Detected</td>
</tr>
<tr>
<td>hR HU (WHO 80/505)</td>
<td>100–2500 IUL</td>
<td>Not Detected</td>
</tr>
<tr>
<td>PL</td>
<td>0.1 mg/mL</td>
<td>Not Detected</td>
</tr>
<tr>
<td>TSH (WHO 80/558)</td>
<td>25–1000 IUL</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

The specificity towards other structural forms of prolactin, including macroprolactin, has not been determined.

Table: OD (450 nm) 4.3

6.5
6.0
5.5
5.0
4.5
4.0
OD 0.0

1. OD 1
2. OD 2
3. Mean OD
4. Value (µIU/mL)

A 0.081 0.079 0.080 0
B 0.099 0.102 0.101 20
C 0.181 0.182 0.182 100
D 0.477 0.481 0.479 400
E 0.769 0.771 0.760 600
F 1.858 2.016 1.937 3200
Unknown 0.442 0.469 0.456 383.458

1. Three samples were assayed ten times each on the same microplate reader at 450 nm.
2. The results (in µIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>202</td>
<td>14</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>586</td>
<td>68</td>
<td>11.6</td>
</tr>
<tr>
<td>3</td>
<td>1220</td>
<td>136</td>
<td>10.3</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in µIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>237</td>
<td>16</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>589</td>
<td>85</td>
<td>14.4</td>
</tr>
<tr>
<td>3</td>
<td>1725</td>
<td>277</td>
<td>13.3</td>
</tr>
</tbody>
</table>

RECOVERY
Sputtered samples were prepared by adding defined amounts of prolactin to three patient serum samples. The results are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked 85</td>
<td>125</td>
<td>46.5</td>
<td></td>
</tr>
<tr>
<td>2 Unspiked 70</td>
<td>100</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td>3 Unspiked 85</td>
<td>125</td>
<td>71.4</td>
<td></td>
</tr>
</tbody>
</table>

LINEARITY
Three patient serum samples were diluted with calibrator A.

The results (in µIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>262</td>
<td>146</td>
<td>55.2</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>73</td>
<td>83.1</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>37</td>
<td>90.2</td>
</tr>
</tbody>
</table>

EXPECTED NORMAL VALUES
For all clinical assays each laboratory should collect data and establish their own range of expected normal values.

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

TYPICAL TABULATED DATA
Sample data only. Do not use to calculate results.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.081</td>
<td>0.079</td>
<td>0.080</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.099</td>
<td>0.102</td>
<td>0.101</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>0.181</td>
<td>0.182</td>
<td>0.182</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>0.477</td>
<td>0.481</td>
<td>0.479</td>
<td>400</td>
</tr>
<tr>
<td>E</td>
<td>0.769</td>
<td>0.771</td>
<td>0.760</td>
<td>600</td>
</tr>
<tr>
<td>F</td>
<td>1.858</td>
<td>2.016</td>
<td>1.937</td>
<td>3200</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.442</td>
<td>0.469</td>
<td>0.456</td>
<td>383.458</td>
</tr>
</tbody>
</table>

REFERENCES

SYMBOLS
- = insufficient for testing
- = not tested