4. Recovery
Known quantities of prolactin were added to a serum that contained a low concentration of Prolactin.
WARRANTINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive
   for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test
   method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These
   reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease
   Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This test kit is USA FDA exempt product.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be
   mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as
   following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield
   invalid data.

SPECIMEN COLLECTION HANDLING
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up
   to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store
at room temperature (18-26°C).

ASSAY PROCEDURE
Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder
2. Pipet 50 μl of prolactin standards, control and patient's sera.
3. Add 100 μl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 30 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 μl of 1X wash buffer.
   Blot on absorbance paper or paper towel.
6. Add 100 μl of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50 μl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS
The standard curve is constructed as follows:
1. Check prolactin standard values on each standard vial. This value might vary from lot to lot. Make sure you check
   the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the standards (vertical axis) versus the standard
   concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or
   unknown sample.

Example of a Standard Curve

<table>
<thead>
<tr>
<th>OD 450 nm</th>
<th>Conc. ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>0.024</td>
</tr>
<tr>
<td>Std 2</td>
<td>0.217</td>
</tr>
<tr>
<td>Std 3</td>
<td>0.372</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.809</td>
</tr>
<tr>
<td>Std 5</td>
<td>1.504</td>
</tr>
<tr>
<td>Std 6</td>
<td>2.851</td>
</tr>
</tbody>
</table>

EXPECTED VALUES
It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local
population. The following values for prolactin may be used as initial guideline ranges only:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Normal Range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>2-17</td>
</tr>
<tr>
<td>Females</td>
<td>3-25</td>
</tr>
<tr>
<td>Pregnancy 3rd trimester</td>
<td>95-480</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE TEST
1. The test results obtained using this kit are for research use only and are not intended to be used as a part of any
   official diagnosis.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS
1. Correlation with a Reference ELISA kit:
   A total of 110 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean ng/ml</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>33.2</td>
<td>2.27</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>15.7</td>
<td>0.75</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>4.2</td>
<td>0.24</td>
<td>5.8</td>
</tr>
</tbody>
</table>

2. Precision
   Intra-Assay

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean ng/ml</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>30.5</td>
<td>2.7</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>14.5</td>
<td>0.98</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4.3</td>
<td>0.3</td>
<td>6.9</td>
</tr>
</tbody>
</table>

3. Sensitivity
   The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the
   same run.

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean ng/ml</th>
<th>Standard Deviation</th>
<th>Mean + 2SD (Sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Standard</td>
<td>20</td>
<td>0.126</td>
<td>0.208</td>
<td>0.334 ng/ml</td>
</tr>
</tbody>
</table>