1. **Intended use**

For IN VITRO determination of serum and plasma rat prolactin level (rPRL).

2. **Principle of the method**

The rat PRL assay is based on the competition between the PRL of the rat sample and a $^{125}$I-labelled rat PRL tracer for binding to a highly specific rabbit polyclonal antibody Ab, according to the following equation:

$$ \frac{\text{PRL}}{\text{rPRL}-\text{Ab}} \times \frac{\text{FREE}}{\text{BOUND}} \times 100 $$

Since the concentrations of $^{125}$I-PRL and Ab are constant, the advancing state of the equation depends on the concentration of rat PRL in the sample. After incubation, separation of bound from free is achieved by the PEG-second antibody method. The radioactive bound fraction is precipitated by centrifugation and counted in a gamma counter.

Rat sample concentrations are read from a calibration curve and the results are expressed in ng/ml.

3. **Warnings and precautions**

For in vitro diagnostic use

It must be handled by specialized staff.

Good laboratory and safety practices are advisable.

**Warning:**

1. This kit contains $^{125}$I emitting X and γ ionizing rays.

2. This radioactive material may be received, acquired, possessed and used only by persons in clinical or hospital laboratories who are authorized by competent authorities and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, transfer, the waste disposal and the people protection are subject to the State and local regulations.

3. Use impermeable gloves and appropriate protection clothes.

**Warning:**

1. Some components contain sodium azide (<1 g/l). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On use, close all reagents vials and bottles and replace these at 2-8°C or -20°C.

1. After use, close all reagents vials and bottles and replace these at 2-8°C or -20°C.

4. **Reagents, preparation and storage**

All reagents are ready for use, except the calibrators (0-5) and control. Stored at 2-8°C, the material can be used up to the expiration date printed on each label. Before use, reconstitute the content of the calibrator 0 with 1 ml of deionized water and the content of the other calibrators (1-5) and control with 0.5 ml of deionized water. Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. If not used immediately after reconstitution, the solutions are stable for two weeks at 2-8°C or for longer period if stored at -20°C.

After use, close all reagents vials and bottles and replace these at 2-8°C or -20°C.

4.1. **Vial 1 (11 ml)** $^{125}$I-labelled rat PRL in buffer with a stabilizer and a preservative (sodium azide < 1 g/l). The vial contains a maximum of 170 KBq (4.6 µCi) at the date of production.

4.2. **Ab Rat PRL**

1. Vial (11 ml, blue) highly specific rabbit antiserum anti native rat PRL diluted in buffer with a stabilizer, a preservative (NaN$_3$ < 1 g/l) and a blue dye.

4.3. **CAL 0**

1. Vial lyophilized horse serum containing preservatives (NaN$_3$ < 1 g/l) and calibrated with sera from hypox rats. The CAL 0 must also be used to dilute samples with concentrations above the higher calibrator (CAL 5).

4.4. **CAL 1-5**

5 vials lyophilized rat PRL supplied in horse serum containing preservatives (NaN$_3$ < 1 g/l). The concentrations of the different calibrators are printed on the vial labels and expressed in ng/ml of highly purified rPRL (purity: > 98 % m/m).

Seeing the lack of international references for rPRL we have established a QC procedure which guarantees batch to batch reproducibility for standard curve calibration.

4.5. **CONTROL**

1 vial lyophilized rat PRL supplied in horse serum containing preservatives (NaN$_3$ < 1 g/l). The control has to be assayed along with the samples and the result compared to the range printed on the vial label.

4.6. **SOLN PEG Ab 2**

2 bottles (110 ml/bottle) of sheep anti-rabbit immunoglobulins mixed with a cellulose PEG solution, a stabilizer and a preservative (NaN$_3$ < 1 g/l).

5. **Material required but not provided**

- bench surfaces protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers appropriately labelled and designed as suitable for solid or liquid radioactive materials and biological materials.
- manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination.
- repeater pipettes (Eppendorf type)
- incubator or water bath
- vacuum pump connected through a trap for aspiration
- centrifuge or magnetic plate
- a vortex mixer
- a calibrated gamma scintillation counter
- appropriate graph paper for plotting the results.

6. **Methodology**

6.1. **Collection and handling of serum or plasma samples**

The blood sample may be collected either into dry tubes or in the presence of anticoagulant (EDTA/CITRATE). If heparin is used, only the minimum required to avoid clotting should be added.

After separation from the red blood cells, plasma or serum samples may be assayed immediately, within 24 hours if stored at 2-8°C or later, after periods as long as several months, if stored at -20°C. Repeated freezing and thawing must be avoided.

6.2. **Assay procedure**

Do not mix reagents of different lots.

Bring the different components of the kit to room temperature prior to use. Perform the assay in duplicates. Calibrators, control and samples must be assayed at the same time. Follow strictly the different steps of the procedure and use interchangeable tips.

Label the tubes for T ("Total count"), NSB, calibrators, control and samples.

1. **NSB**

Pipette 50 µl of calibrator 0 and 100 µl of distilled water into the corresponding tubes.

2. **Calibrators**

Pipette 50 µl of each calibrator into the corresponding tubes.

3. **Samples and control**

Pipette 50 µl of each sample and control into the corresponding tubes.

4. **Add 100 µl of Rat PRL $^{125}$I tracer to each tube. “Total count” tubes do not participate to the following steps.

5. **Add 100 µl of antiserum (Ab Rat PRL) to each tube except NSB and “Total count” tubes. Mix all tubes with a vortex mixer.

6. **Incubate overnight at room temperature.

7. **Maintain the SOLN PEG Ab 2** in suspension by magnetic stirring and add 2 ml to each tube except the “Total Count” tubes.

8. **Mix all tubes with a vortex mixer and incubate for 30 minutes at room temperature without further mixing.

9. **Centrifuge all tubes, except « Total count » tubes, at 1500 g for at least 15 minutes.

10. **Carefully aspirate the supernatants.

11. **Count the radioactivity precipitated in each tube for at least 60 seconds.

6.3. **Data processing**

Determine the mean count rate for each set of duplicate tubes.

Calculate the ratio B/B0 as follows:

$$ \text{B/B0} = \frac{\text{cal or smp cpm} - \text{NSB cpm}}{\text{B0 (Cal 0) cpm} - \text{NSB cpm}} \times 100 $$

Draw the standard curve on semilogarithmic paper by plotting the ratio B/B0 % (linear scale) obtained for each calibrator versus its respective concentration expressed in ng/ml (logarithmic scale).

Rat PRL concentrations in samples may be read directly from the standard curve.
6.4 Example of a typical assay

<table>
<thead>
<tr>
<th>Contents (ng/ml)</th>
<th>cpm 1st duplicate</th>
<th>cpm 2nd duplicate</th>
<th>Mean count rate</th>
<th>B/B0 %</th>
<th>ratPRL conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Total counts&quot;</td>
<td>-</td>
<td>39575</td>
<td>39815</td>
<td>39695</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>-</td>
<td>2029</td>
<td>2125</td>
<td>2077</td>
<td>-</td>
</tr>
<tr>
<td>CAL 0 (B0)</td>
<td>0</td>
<td>24762</td>
<td>25306</td>
<td>25034</td>
<td>100</td>
</tr>
<tr>
<td>CAL 1</td>
<td>6</td>
<td>23457</td>
<td>24051</td>
<td>23754</td>
<td>94.4</td>
</tr>
<tr>
<td>CAL 2</td>
<td>27</td>
<td>20439</td>
<td>20475</td>
<td>20457</td>
<td>80.1</td>
</tr>
<tr>
<td>CAL 3</td>
<td>65</td>
<td>16480</td>
<td>16454</td>
<td>16467</td>
<td>62.7</td>
</tr>
<tr>
<td>CAL 4</td>
<td>130</td>
<td>12851</td>
<td>12367</td>
<td>12609</td>
<td>45.9</td>
</tr>
<tr>
<td>CAL 5</td>
<td>250</td>
<td>8728</td>
<td>8618</td>
<td>8673</td>
<td>28.7</td>
</tr>
<tr>
<td>CONTROL</td>
<td>30.4 – 50.2</td>
<td>18303</td>
<td>19433</td>
<td>18893</td>
<td>73.2</td>
</tr>
<tr>
<td>Sample 1</td>
<td>-</td>
<td>21146</td>
<td>21736</td>
<td>21441</td>
<td>84.3</td>
</tr>
<tr>
<td>Sample 2</td>
<td>-</td>
<td>17360</td>
<td>17372</td>
<td>17366</td>
<td>66.6</td>
</tr>
<tr>
<td>Sample 3</td>
<td>-</td>
<td>12525</td>
<td>12573</td>
<td>12549</td>
<td>45.6</td>
</tr>
</tbody>
</table>

Example of typical assays, do not use for calculations

7. Expected normal values

It is recommended that each laboratory establishes its own reference values. Concentrations range for normal male subjects from 6.64 to 23.26 ng/ml and for normal female subjects from 13.7 to 23.3 ng/ml in our preliminary studies.

8. Limitation of the procedure

Do not use strongly lipemic, haemolyzed, icteric or turbid specimens

9. Quality control

Use the control provided for each assay. If, in normal using conditions, the control is out the acceptable range, the sample results can't be validated. Please contact the manufacturer.

10. Performance characteristics

10.1 Specificity

The assay was found to be specific for rPRL. PRL from other species can crossreact partially in this assay. The weak cross-reactivities measured with other rat pituitary hormones are due to contamination of the hormonal preparation as checked by radioreceptor assay.

10.2 Analytical sensitivity

The minimum detectable concentration of rat PRL has been assayed at 0.5 ng/ml and corresponds to the concentration given by two standard deviations below the mean cpm of 20 replicate determinations of the zero calibrator.

10.3 Imprecision

<table>
<thead>
<tr>
<th>Mean value (ng/ml)</th>
<th>Repeatability Within assay variation (%CV) 18 replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.4</td>
</tr>
<tr>
<td>Sample 2</td>
<td>76.5</td>
</tr>
<tr>
<td>Sample 3</td>
<td>144.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean value (ng/ml)</th>
<th>Reproducibility Between assay variation (%CV) 8 separate assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.4</td>
</tr>
<tr>
<td>Sample 2</td>
<td>76.5</td>
</tr>
<tr>
<td>Sample 3</td>
<td>144.5</td>
</tr>
</tbody>
</table>

11. Bibliography

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Catalogue number 07C-74102
Package insert /date of issue : 2003-12-17