Prolactin IRMA (CT)

Radioimmunoassay (CT) for the quantitative measurement of human prolactin (PRL) in serum and plasma.

REF  MG12161

Σ  96

2-8 °C

EU: IVD  CE
Read entire protocol before use.

Prolactin IRMA (CT)

I. INTENDED USE

Immunoradiometric assay kit for the in vitro quantitative measurement of human prolactin (PRL) in serum and plasma.

II. GENERAL INFORMATION

A. Proprietary name: Prolactin IRMA (CT)

B. Catalog number: MG12161

III. CLINICAL BACKGROUND

A. Biological activities

Prolactin (PRL) is a polypeptide hormone (molecular weight 20,000 Da) secreted by the pituitary gland, which plays a key role in the development of the mammary gland, the production and secretion of milk and the control of male and female gonadal functions. Prolactin secretion is under hypothalamic control exerted directly by dopamine, several prolactin releasing factors (PRF) and perhaps VIP (vasoactive intestinal polypeptide) or a closely related peptide. TRH also acts directly at the pituitary level to stimulate prolactin release but its physiological role in the control of prolactin secretion has not been established yet. Several neuroendocrine factors, involving serotoninergic or noradrenergic pathways are also involved in the control of prolactin secretion. The plasma concentration of prolactin increases in various physiological situations such as stress, pregnancy and lactation. Physiological levels fluctuate according to a nycthemeral rhythm, a significant rise being observed at night. Drugs with anti-dopamine activity (psychotropic agents) and ovulatory suppressants, increase prolactin secretion.

B. Clinical application

- **Prolactinoma**: Circulating prolactin levels are elevated in patients with a prolactin secreting pituitary adenoma. Amenorrhea and impotence are characteristic clinical symptoms in such cases.

- **Other pituitary diseases**: Increased prolactin levels are also observed in 5% to 20% of patients with acromegaly and when pituitary control by the hypothalamus is suppressed (pituitary stalk section). Decreased PRL levels may be observed in cases of complete destruction of the pituitary as in Sheehan's syndrome.

- **Galactorrhea and amenorrhea**: The measurement of the prolactin levels in serum is a useful test in the differential diagnosis of galactorrhea and amenorrhea.
IV. PRINCIPLES OF THE METHOD

The Prolactin IRMA (CT) is an immunoradiometric assay based on coated-tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with 125I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity.

V. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
<th>Colour Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBES</td>
<td>2 x 48</td>
<td>orange</td>
<td>Ready for use</td>
</tr>
</tbody>
</table>

Tubes coated with anti-PRL (monoclonal antibodies)

| TRACER           | 1 vial   | red         | Ready for use    |

Anti-PRL-125I (monoclonal antibodies) in TRIS Buffer

| CAL 6 LYO       | 1 vial lyophil. | yellow | Add 2.0 ml distilled water |

Zero Calibrator in bovine serum with thymol

| CAL N LYO       | 5 vials lyophil. | yellow | Add 0.5 ml distilled water |

Calibrators 1-5 in bovine serum with thymol (see exact values on vial labels)

| WASHER BUF CONC | 1 vial 10 ml | brown | Dilute 70 x in distilled water (use a magnetic stirrer). |

Wash solution (TRIS-EDTA)

| CONTROL N LYO   | 2 vials lyophil. | silver | Add 0.5 ml distilled water |

Controls 1 and 2 in human plasma with thymol

Note: 1. Use the zero calibrator for sera dilutions.
2. 1 ng of the calibrator preparation is equivalent to 29 µIU NIBSC 3rd IS 84/500.
3. Conversion factor: ng/ml x 29 = µUI / ml

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 25 µl, 200 µl, 500 µl and 2 ml. (The use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. 5 ml automatic syringe (Cornwall type) for washing
6. Aspiration system (optional).
7. Any gamma counter capable of measuring 125I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Calibrators: Reconstitute the zero calibrator with 2 ml distilled water and the other calibrators with 0.5 ml distilled water.
B. Controls: Reconstitute the controls with 0.5 ml distilled water.
C. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 7 days at 2-8°C. For longer storage periods, aliquots should be made and kept at –20°C for 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- If the test is not run within 24 hours, storage at –20°C is recommended.
- Do not use haemolysed samples.
- Serum or plasma (EDTA and heparin) provides similar results. Y (serum) = 1.09x (hep. plasma) + 0.05  r = 0.95  n = 30
- Y (serum) = 0.96x (EDTA plasma) + 0.14  r = 0.98  n = 30

IX. SPECIMEN COLLECTION AND PREPARATION

β. Serum and plasma must be kept at 2 to 8°C.
β. If the test is not run within 24 hours, storage at –20°C is recommended.
β. Avoid subsequent freeze-thaw cycles.
β. Do not use haemolysed samples.
β. Serum or plasma (EDTA and heparin) provides similar results.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times.

Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, samples and controls and dispense 25 µl of each into the respective tubes.
3. Dispense 200 µl of anti-PRL-125I tracer into each tube, including the uncoated tubes for total counts.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hours at room temperature.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash the tubes with 2 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
8. Aspirate (or decant) the content of each tube (except total counts).
9. Let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
10. Count the tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semi-logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of PRL (abscissa) and draw a calibration curve through the calibration points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

<table>
<thead>
<tr>
<th>PRL-IRMA</th>
<th>cpm</th>
<th>B/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count</td>
<td>135774</td>
<td>100</td>
</tr>
<tr>
<td>Calibrator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0 ng/ml</td>
<td>224</td>
<td>0.16</td>
</tr>
<tr>
<td>2.8 ng/ml</td>
<td>1266</td>
<td>0.93</td>
</tr>
<tr>
<td>9.4 ng/ml</td>
<td>3401</td>
<td>2.5</td>
</tr>
<tr>
<td>30.0 ng/ml</td>
<td>8124</td>
<td>5.98</td>
</tr>
<tr>
<td>80.0 ng/ml</td>
<td>17778</td>
<td>13.09</td>
</tr>
<tr>
<td>133.0 ng/ml</td>
<td>25312</td>
<td>18.64</td>
</tr>
</tbody>
</table>

Detection range: 0.35 to 133 ng/ml

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average counts at zero binding, was 0.35 ng/ml.

B. Specificity

Cross-reactive hormones were added to a low and to a high PRL value calibrator. The apparent PRL response was measured.

C. Precision

The potentially interfering effects of hemoglobin at 7.5 mg/ml and of bilirubin at 0.2 mg/ml have been evaluated. The results of this test do not demonstrate any significant interference (see the table below).

D. Accuracy

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added PRL (ng/ml)</th>
<th>Recovered PRL (ng/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>1.8</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.8</td>
<td>98.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.5</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>45.6</td>
<td>91.2</td>
</tr>
</tbody>
</table>

E. Time Delay

As shown below, assay results remain accurate even when a sample is dispensed up to 60 minutes after the calibrator has been added to the coated tubes.

F. Hook effect

A serum sample with a concentration of 18000 ng/ml PRL gives a signal above the highest calibrator concentration.

XIV. LIMITATIONS

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed in case of the presence of heterophile antibodies. Carefully evaluate the results of these specimens.
- Samples should rely on Good Laboratory Practises.

XV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.
**XVI. REFERENCE INTERVALS**

The values provided below are given only for guidance; each laboratory should establish its own normal range of values. PRL concentrations were measured in serum samples obtained from different categories of healthy subjects.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Number of subjects</th>
<th>Mean (ng/ml)</th>
<th>Range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>97</td>
<td>4.8</td>
<td>1.8 - 15.9</td>
</tr>
<tr>
<td>Pre-menopausal women</td>
<td>95</td>
<td>8.6</td>
<td>2.7 - 19.7</td>
</tr>
<tr>
<td>Post-menopausal women</td>
<td>47</td>
<td>6.1</td>
<td>1.9 - 17.9</td>
</tr>
</tbody>
</table>

**XVII. PRECAUTIONS AND WARNINGS**

**Safety**

For in vitro diagnostic use only. This kit contains 125I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals. All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes. Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection. The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious. Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

**XVIII. BIBLIOGRAPHY**


**XIX. SUMMARY OF THE PROTOCOL**

<table>
<thead>
<tr>
<th>TOTAL COUNTS</th>
<th>CALIBRATORS</th>
<th>SAMPLE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>ml</td>
<td>ml</td>
</tr>
<tr>
<td>Calibrators (0-5)</td>
<td>0.025</td>
<td>-</td>
</tr>
<tr>
<td>Samples</td>
<td>0.200</td>
<td>0.200</td>
</tr>
<tr>
<td>Tracer</td>
<td>0.025</td>
<td>0.200</td>
</tr>
<tr>
<td>Incubation</td>
<td>2 hours</td>
<td>room</td>
</tr>
<tr>
<td>Separation</td>
<td>aspirate (or decant)</td>
<td></td>
</tr>
<tr>
<td>Washing solution</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Separation</td>
<td>aspirate (or decant)</td>
<td></td>
</tr>
<tr>
<td>Counting</td>
<td>Count tubes</td>
<td>60 seconds</td>
</tr>
</tbody>
</table>

Date of issue: 110218/3

Revision date: 2016-02-11
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.° Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.°: / Lotto n.: / Αριθμός-Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostic In vitro. / Ιατρική συσκευή για Ιν-Βίτρο Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED. 
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. 
Voir MATERIEL FOURNI pour les symbôles des composants du kit. 
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. 
Para símbolos dos componentes del kit ver MATERIAIS FORNECIDOS. 
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. 
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany
Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com

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