Instructions for Use

CBG RIA

Radioimmunoassay for the quantitative determination of CBG (Transcortin) in human serum.

REF      MG13061
Σ      96

2-8°C

EU: IVD  CE

IBL INTERNATIONAL GMBH
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com
Read entire protocol before use.

**CBG RIA**

**I. INTENDED USE**

Radioimmunoassay for the quantitative measurement of human Transcortine or Corticosteroid Binding Globulin (CBG) in serum.

**II. GENERAL INFORMATION**

A. Proprietary name : CBG RIA

B. Catalog number : MG13061 : 96 tests

**III. CLINICAL BACKGROUND**

A. **Biological activity**

Transcortin or corticosteroid-binding globulin (CBG) is a plasma α₁-glycoprotein with a molecular weight of approximately 52000 Dalton. It contains a single steroid-binding site with an affinity (at 37°C) for cortisol of $3.10^{7}$ M⁻¹ and a somewhat lower affinity for progesterone. Since the plasma concentration of transcortin varies between 0.4 and 2.5 10⁻⁶ M, the major fraction of cortisol in plasma is bound to this protein. This transcortin-bound cortisol is considered to be biologically inactive, whereas the unbound cortisol constitutes the active form of cortisol. The active fraction of plasma cortisol will thus depend on the concentration of transcortin.

B. **Clinical applications**

The plasma concentration of transcortin shows little or no diurnal variation and no marked differences are observed in adult subjects according to age, sex or menstrual cycle. In umbilical cord blood, however, transcortin is present at half of the normal adult level and prepubertal children have somewhat higher levels than adults. Estrogen therapy or estrogen impregnation during pregnancy causes a very marked increase of the transcortin concentration. Decreased levels of transcortin are observed in several conditions: hypoproteinemia, Cushing's syndrome or corticoid treatment and in some cases of vitamin B₁₂ deficiency. Extremely low levels of transcortin have been reported in a few patients with septic shock. Furthermore, a rare inherited form of hypotranscortinemia has been described.

The most important clinical application of transcortin measurements consists of the interpretation of cortisol levels, since it allows to assess the unbound cortisol concentration, which is biologically active. Indeed, the concentration of unbound cortisol can be calculated from the concentration of total cortisol and that of transcortin on the basis of mass action. The results of this method correlate well with those obtained by centrifuged ultrafiltrations.
IV. PRINCIPLES OF THE METHOD

A fixed amount of 125I labelled CBG competes with the CBG to be measured present in the sample or in the calibrator for a fixed amount of anti-CBG antibody sites, which are bound to the goat anti mouse (GAM) antibodies immobilized to the wall of a polystyrene tube. After 2 hours incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of working wash solution and aspirated again. A calibration curve is plotted and the CBG concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Reagents</th>
<th>96 Test Kit</th>
<th>Colour Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBES</td>
<td>2 x 48</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>TRACER</td>
<td>1 vial</td>
<td>red</td>
<td>Ready for use</td>
</tr>
<tr>
<td>CAL 0 calibrator</td>
<td>1 vial</td>
<td>yellow</td>
<td>Add 3 ml distilled water</td>
</tr>
<tr>
<td>CAL N calibrator</td>
<td>1 vial</td>
<td>blue</td>
<td>Ready for use</td>
</tr>
<tr>
<td>ANTISERUM</td>
<td>1 vial</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>1 vial</td>
<td>brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer).</td>
</tr>
<tr>
<td>CONTROL N control</td>
<td>2 vials</td>
<td>silver</td>
<td>Add 0.5 ml distilled water</td>
</tr>
</tbody>
</table>

To the best of our knowledge, no international reference material exists for this parameter.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 100 μl, 500 μl, 1 ml, 3 ml and 5 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Disposable polystyrene tubes (12 x 75 mm)
4. Vortex mixer
5. Tube shaker (400 rpm)
6. Magnetic stirrer
7. 5 ml automatic syringe (Cornwall type) for washing
8. Aspiration system (optional)
9. Any gamma counter capable of measuring 125I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Calibrators: Reconstitute the zero calibrator with 3 ml distilled water and the other calibrators with 1 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 maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 48 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.
- After thawing, the samples should be mixed and centrifuged.
- The samples have to be diluted 25 times in Dilution Buffer. Recommended procedure: 100 μl serum + 2.4 ml Dilution Buffer.

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. Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. 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 the determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls and diluted samples and dispense 100μl of each into the respective tubes.
3. Dispense 100 μl of 125I labelled CBG into each tube, including the tubes for total counts.
4. Dispense 100 μl of CBG antiserum into each tube (except total counts).
5. Shake the tube rack gently by hand to liberate any trapped air bubbles.
6. Incubate for 2 hour at room temperature with continuous shaking at 400 rpm.
7. 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.
8. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate. Avoid foaming during the addition of the Working Wash solution.
9. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
10. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Using a 3 cycle semi-logarithmic or log-log graph paper, plot the (B/B0(%) values for each calibrator point as a function of the CBG concentration of each calibrator point. Reject obvious outliers.
3. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
4. By interpolation of the sample (B/B0 (%)) values, determine the CBG concentrations of the samples from the calibration curve.
5. The concentrations read on the calibration curve for the samples and controls must be multiplied by 25 (dilution factor).
6. For each assay, the percentage of total tracer bound in the absence of unlabelled CBG (B0/T) must be checked.
Calculation of unbound cortisol

In human serum cortisol is bound to transcortin, and, in addition there is some weak non-saturable binding to albumin. These simultaneous binding equilibria can be represented by the following equation:

\[ U'K(1+N) + U(1+N) + K(T-C) - C = 0 \]

In this equation, \( U \) represents the molar concentration of unbound cortisol, \( C \) the molar concentration of total cortisol and \( T \) the concentration of transcortin. \( K \) corresponds to the affinity of transcortin for cortisol at 37°C and \( N \) to the proportion of albumin-bound to unbound cortisol. This equation can be solved for \( U \) in the following way:

\[ U = \frac{Z^2 + C}{(1 + N)K} \]

or quantitatively, assuming a value for \( K \) of \( 3 \times 10^{-7} \text{ M}^{-1} \) and a value for \( N \) of 1.74 and expressing \( U, C \) and \( T \) as \( \mu \text{M} \).

\[ U = \sqrt{Z^2 + 0.0122C} - Z \mu \text{M} \]

where \( Z = \frac{1}{2K} + \frac{T-C}{2(1+N)} \mu \text{M} \)

To convert concentrations of cortisol in \( \mu \text{g}% \) or in ng/ml to \( \mu \text{M} \) values, divide by 52. The obtained value of \( U \) (in \( \mu \text{M} \)) can be converted to concentrations of transcortin in \( \mu \text{g/ml} \) to \( \% \) by multiplication with 36.2 or ng/ml by multiplication with 362.

Example of calculation: let’s suppose that the obtained transcortin and total cortisol levels are respectively of 40 \( \mu \text{g/ml} \) and 130 ng/ml.

- Transcortin levels in \( \mu \text{g/ml} \): \( \frac{40}{52} = 0.77 \mu \text{M} \)
- Total cortisol levels in \( \mu \text{g/ml} \): \( \frac{130}{362} = 0.36 \mu \text{M} \)
- \( Z = \frac{0.0167 + 0.182(0.77-0.36)}{0.09} \mu \text{M} \)
- \( U = \sqrt{0.021)(0.09)} \mu \text{M} \)
- Concentration of unbound cortisol in ng/ml: 0.021 x 362 = 7.8 ng/ml.

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>Substance</th>
<th>CBG (µg/ml)</th>
<th>cpm</th>
<th>B/Bn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count</td>
<td>42523</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator</td>
<td>0.00 µg/ml</td>
<td>17216</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>0.44 µg/ml</td>
<td>15262</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>0.81 µg/ml</td>
<td>13081</td>
<td>80.3</td>
</tr>
<tr>
<td></td>
<td>1.50 µg/ml</td>
<td>10162</td>
<td>61.6</td>
</tr>
<tr>
<td></td>
<td>2.20 µg/ml</td>
<td>8292</td>
<td>48.6</td>
</tr>
<tr>
<td></td>
<td>4.00 µg/ml</td>
<td>4429</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>8.00 µg/ml</td>
<td>2633</td>
<td>11.3</td>
</tr>
</tbody>
</table>

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 below the average counts at zero binding, was 0.26 µg/ml.

B. Precision

<table>
<thead>
<tr>
<th>Substance</th>
<th>CBG (µg/ml)</th>
<th>Interferent mg/dl</th>
<th>% Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>55.5</td>
<td>500</td>
<td>-5.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>125.5</td>
<td>500</td>
<td>0.0</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>58.5</td>
<td>500</td>
<td>3.9</td>
</tr>
<tr>
<td>54.0</td>
<td>1000</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>55.5</td>
<td>20</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>125.5</td>
<td>20</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>58.5</td>
<td>20</td>
<td>7.7</td>
<td></td>
</tr>
</tbody>
</table>

XIV. 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.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Range (*) (µg/ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>22 - 55</td>
<td>16</td>
</tr>
<tr>
<td>Women</td>
<td>40 - 154</td>
<td>43</td>
</tr>
</tbody>
</table>

(*) The range is based on 2.5 % and 97.5 % percentiles.
XVI. 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 radionuclides. 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. 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.

XVII. BIBLIOGRAPHY

1. BRIEN T.G., 1980
   Free cortisol in human plasma.
   Gorm. Metab. Res. 12, 643-650

2. BRIEN T.G., 1981
   Human corticosteroid binding globulin.
   Clin. Endocrinol. 14, 193-212

3. DAUGHADAY W.H., 1958
   Binding of corticosteroid by plasma proteins. Corticosteroid-binding globulin activity in normal human beings and in certain disease states.
   Arch. int. Med., 101, 286

   Protein-binding of corticosteroid studies by gel filtration.
   J. Clin. Invest. 41, 816-827

5. FAICT D. and DE MOOR P., 1984
   Use of monoclonal antibodies in a RIA for human transcortin.
   Clin. Chem. 30, 369-372

6. HEYNS W., COOLENS J.L., VAN BAELEN H., and DE MOOR P., 1982
   Dosage et signification du cortisol libre dans le sang.
   Journal de Biophysique et Médecine Nucléaire, in press.

7. PARTRIDGE W.M., 1981
   Transport of protein-bound hormones into tissues in vivo.
   Endocrine Reviews, 2, 103-123

8. ROBIN P., PREDINE J. and MILGROM T., 1978
   Assay of unbound cortisol in plasma.

   Serum depletion of cortisolsteroid binding-activities, an early marker of human sceptic shock.

    Purification and properbess of transcortin, the cortisol binding globulin, from patients with cancer of the prostate.
    Cancer Chemotherapy Reports, 16, 329-334

11. SLAUWHITE W.R. and SANDBERG A.A., 1974
    Transcortin : a corticosteroid-binding protein of plasma.
    J. Clin. Invest. 38, 384-391

12. VAN BAELEN H. and DE MOOR P., 1974
    Immunochemical quantitation of human transcortin.
    J. Clin. Endocrinol and Metab. 39, 160-163

    Transcortin Leuven : a variant of human corticosteroid-binding globulin with decreased cortisol binding affinity.

14. WESTPHAL U., 1971
    Steroid-protein interactions.
    Springer Verlag.

15. WESTPHAL U., 1983
    Corticosteroid-binding globulin. A review of some recent aspects.

16. ROSNER W., 1972
    Recent studies on the binding of cortisol in serum.
    J. Steroid Biochem., 3, 531-542

XVIII. SUMMARY OF THE PROTOCOL

<table>
<thead>
<tr>
<th></th>
<th>TOTAL COUNTS</th>
<th>CALIBRATORS</th>
<th>SAMPLE(S) CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>ml</td>
<td>ml</td>
</tr>
<tr>
<td>Calibrators (0 to 6)</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Samples. Controls</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Tracer</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-CBG</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Incubation</td>
<td>2 hour at room temperature with continuous shaking at 400 rpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separation</td>
<td>-</td>
<td>Aspirate (or decant)</td>
<td></td>
</tr>
<tr>
<td>Working Wash solution</td>
<td>-</td>
<td>Aspirate (or decant)</td>
<td></td>
</tr>
<tr>
<td>Counting</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Count tubes for 60 sec

Revision nr : 160822/1

Revision date: 2016-08-22
Symbols / Symbole / Symboles / 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></td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td></td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td></td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</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. / Manténase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / 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 symboles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do 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

Symbols Version 4.5 / 2015-12-07