



CBG-RIA-CT

KIR1809

History

Summary of change:

Previous Version: 200122-1	Current Version: 230113
<i>V. REAGENTS PROVIDED</i> Dilution Buffer : 110 ml	<i>V. REAGENTS PROVIDED</i> Dilution Buffer : 108 ml Removal the column for “Color code”
	<i>X. Procedure – A. Handling notes</i> Adding of the following sentence : “Attention: Performance of the kit was defined based on samples tested in duplicate, it is thus important to use the kit as recommended in the IFU. For this reason, the volume of dilution buffer provided in the kit is only sufficient to perform the dilution for a duplicate determination of the patient samples.”

Read entire protocol before use.

CBG-RIA-CT

I. INTENDED USE

Radioimmunoassay for the measurement of human Transcortine or Corticosteroid Binding Globulin (CBG) in serum. **For research use only. Not for use in diagnostic procedures.**

II. GENERAL INFORMATION

- A. **Proprietary name :** DIAsource CBG-RIA-CT Kit
- B. **Catalog number :** KIR1809 : 96 tests
- C. **Manufactured by :** DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information in the United States contact :
Immuno-Biological Laboratories, Inc. (IBL-America)

Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com

III. BACKGROUND


Biological activity

Transcortin or corticosteroid-binding globulin (CBG) is a plasma α_1 -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 $3 \cdot 10^7 \text{ M}^{-1}$ and a somewhat lower affinity for progesterone. Since the plasma concentration of transcortin varies between 0.4 and $2.5 \cdot 10^{-6} \text{ M}$, 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.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I 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 18-25°C, 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

Reagents	96 Test Kit	Reconstitution			
 Tubes coated with GAM (Goat anti Mouse)	2 x 48	Ready for use			
<table border="1" data-bbox="119 593 263 645"><tr><td>Ag</td><td>^{125}I</td></tr></table> TRACER: ^{125}I iodine labelled CBG in phosphate buffer with bovine serum albumin and azide (<0.1%)	Ag	^{125}I	1 vial 10.5 ml 89 kBq	Ready for use	
Ag	^{125}I				
<table border="1" data-bbox="119 750 247 788"><tr><td>CAL</td><td>0</td></tr></table> Zero Calibrator in phosphate buffer with bovine serum albumin and azide (<0.1%)	CAL	0	1 vial lyophilised	Add 3 ml distilled water	
CAL	0				
<table border="1" data-bbox="119 878 247 916"><tr><td>CAL</td><td>N</td></tr></table> Calibrators - N = 1 to 6 (see exact values on vial labels) in phosphate buffer with bovine serum albumin and azide (<0.1%)	CAL	N	6 vials lyophilised	Add 1ml distilled water	
CAL	N				
<table border="1" data-bbox="119 1034 311 1072"><tr><td>ANTISERUM</td></tr></table> CBG Antiserum in phosphate buffer with bovine serum albumin and azide (<0.1%)	ANTISERUM	1 vial 10.5 ml	Ready for use		
ANTISERUM					
<table border="1" data-bbox="135 1176 295 1214"><tr><td>DIL</td><td>BUF</td></tr></table> Dilution Buffer: phosphate buffer with bovine serum albumin and azide (<0.1%)	DIL	BUF	1 vial 108 ml	Ready for use	
DIL	BUF				
<table border="1" data-bbox="87 1326 343 1364"><tr><td>WASH</td><td>SOLN</td><td>CONC</td></tr></table> Wash solution (TRIS-HCl)	WASH	SOLN	CONC	1 vial 10 ml	Dilute 70 x with distilled water (use a magnetic stirrer).
WASH	SOLN	CONC			
<table border="1" data-bbox="103 1422 295 1460"><tr><td>CONTROL</td><td>N</td></tr></table> Controls - N = 1 or 2: phosphate buffer with human plasma (diluted 25x), bovine serum albumin and azide (<0.1%) (see exact values on vial labels)	CONTROL	N	2 vials lyophilised	Add 0.5 ml distilled water	
CONTROL	N				

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 ^{125}I 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 kits 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.**
- Avoid hyperlipemic samples.

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 18-25°C 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.
Each tube can only be used once.

Attention: Performance of the kit was defined based on samples tested in duplicate, it is thus important to use the kit as recommended in the IFU. For this reason, the volume of dilution buffer provided in the kit is only sufficient to perform the dilution for a duplicate determination of the patient samples.

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 100 μl of each into the respective tubes.
3. Dispense 100 μl of ^{125}I iodine 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 18-25°C 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. 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 equilibrium can be represented by the following equation :

$$U^2K(1+N) + U(1+N+K(T-C)) - C = 0$$

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 :

$$U = \sqrt{Z^2 + \frac{C}{(1+N)K}} - ZM$$

where in : $Z = \frac{1}{2K} + \frac{T-C}{2(1+N)}M$

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

$$U = \sqrt{Z^2 + 0.0122C} - Z^{\mu M}$$

where in : $Z = 0.0167 + 0.182(T-C)\mu M$

To convert concentrations of cortisol in $\mu g/ml$ or in ng/ml to μM values, divide respectively by 36.2 or 362 to convert concentrations of transcortin in $\mu g/ml$ to μM values, divide by 52. The obtained value of U (in μM) can be converted to $\mu g/ml$ 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 $\mu g/ml$ and 130 ng/ml

· Transcortin levels in μM : $\frac{40}{52} = 0.77\mu M$

· Total cortisol levels in μM : $\frac{130}{362} = 0.36\mu M$

· $Z = 0.0167 + 0.182(0.77-0.36) = 0.09 \mu M$

· $U = \sqrt{0.09^2 + (0.0122 \times 0.36)} - 0.09 = 0.021\mu M$

· Concentration of unbound cortisol in ng/ml : $0.021 \times 362 = 7.8 \text{ ng/ml}$.

XII. TYPICAL DATA

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

Reminder (cfr section XI. 5) : The concentrations read on the calibration curve for the samples and controls must be multiplied by 25 (dilution factor).

CBG	cpm	B/B ₀ (%)
Total count	42523	
Calibrator		
0.00 $\mu g/ml$	17216	100.0
0.44 $\mu g/ml$	15282	89.6
0.81 $\mu g/ml$	13081	80.3
1.50 $\mu g/ml$	10162	61.6
2.20 $\mu g/ml$	8292	48.6
4.00 $\mu g/ml$	4429	21.2
8.00 $\mu g/ml$	2633	11.3

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantification (LoQ) were determined in accordance with the CLSI guideline EP17-A.

The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the tests values. The LoB was calculated to be 0.28 $\mu g/ml$.

The LoD was calculated as described in the guideline. The LoD was calculated to be 1.91 $\mu g/ml$.

The LoQ was calculated by testing 5 samples of low value 10 times in different tests. The LoQ was calculated to be 5.35 $\mu g/ml$ with CV of 20%.

B. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD ($\mu g/ml$)	CV (%)	Serum	N	<X> ± SD ($\mu g/ml$)	CV (%)
A	20	32.5 ± 1.2	3.69	A	10	31.6 ± 1.2	3.6
B	20	80.5 ± 4.4	5.48	B	10	77.9 ± 3.0	4.3

SD: Standard Deviation; CV: Coefficient of variation

C. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. ($\mu g/ml$)	Measured Concent. ($\mu g/ml$)
A	1/8	-	5.5
	1/16	2.75	3.2
	1/32	1.38	1.5
	1/64	0.69	0.69
	1/128	0.34	0.44

Samples were diluted with zero calibrator.

RECOVERY TEST

Sample	added CBG ($\mu g/ml$)	Recovered CBG ($\mu g/ml$)	Recovered (%)
1	0.46	0.4	87.0%
	0.88	1	113.6%
	1.4	1.3	92.9%
	2.1	2.2	104.8%
	4.3	4.1	95.3%
	8.7	9.4	108.0%

D. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to the tubes.

TIME DELAY

Serum $\mu g/ml$	0'	10'	20'	30'
C 1	25.3	30.3	28.8	29.3
C 2	101.8	105.8	106.3	101.5

E. Interferences

Potential interfering substances were tested using the Diasorin CBG-RIA-CT kit. Our acceptance criteria was to obtain an eventual interference of less than 10%. The performances of the kit were not affected by haemoglobin, bilirubin and triglycerides.

Substance	CBG µg/ml	Interferent mg/dl	% Variation
Hemoglobin	27.38	500	4%
	63.44	500	9%
	50.82	500	2%
Triglycerides	69.07	1000	1%
	5.07	1000	9%
	4.66	1000	3%
Bilirubin	27.38	20	4%
	63.44	20	3%
	50.82	20	3%

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.

Identification	Range (*) (µg/ml)	n
Men	22 - 55	16
Women	40 - 154	43

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

XVI. PRECAUTIONS AND WARNINGS

Safety

For research use only. Not for use in diagnostic procedures.

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. 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.

For more information, see Material Safety Data Sheet (MSDS).

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
Calibrators (0 to 6)	-	100	-
Samples, Controls	-	-	100
Tracer	100	100	100
Anti-CBG	-	100	100
Incubation	2 hour at 18-25°C with continuous shaking at 400 rpm		
Separation	-	Aspirate (or decant)	
Working Wash solution		2.0 ml	
Separation		Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

Diasource's Instrumentation Service confirms that the kit is valid for use on the platform Stratec Riamat 300. If you need any additional information, please contact IVDInstrumentation@diasource.be

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Distributed by:



Immuno-Biological Laboratories, Inc. (IBL-America)

8201 Central Ave. NE, Suite P
Minneapolis, MN 55432, USA
Phone: (888) 523-1246
Fax.: (763) 780-2988
Web: www.ibl-america.com
Email: info@ibl-america.com