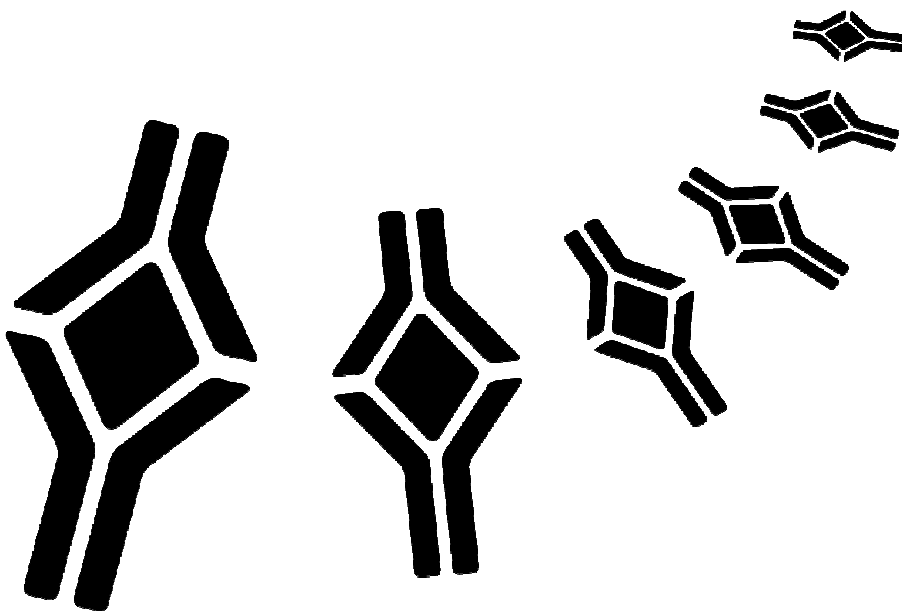


BioVendor

Research
and Diagnostic Products



HUMAN CORTICOSTEROID BINDING GLOBULIN ELISA

Product Data Sheet

Cat. No.: RD192234200R

For Research Use Only

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**➤➤ This kit is manufactured by:
BioVendor – Laboratorni medicina a.s.**

➤➤ Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The RD192234200R Human Corticosteroid Binding Globulin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human corticosteroid binding globulin.

»» Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures total corticosteroid binding globulin in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based. No animal sera are used
- Standard is human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Human corticosteroid binding globulin (CBG, transcortin), also referred to as SerpinA6, belongs to the serpin superfamily. Corticosteroid binding globulin is a 52 kDa secreted α_1 -glycoprotein consisting of 405 amino acids.

Corticosteroid binding globulin is synthesized and secreted by hepatocytes in the liver and is present in glycocorticoid responsive cells. The concentration of corticosteroid binding globulin is regulated by estrogens.

CBG is the major transport protein for progestins and glucocorticoids within the blood. Thus CBG regulates their bioavailability and metabolic clearance and protects them from absorption into cells and degradation by chemicals and enzymes. CBG contains a single steroid binding site with high affinity for cortisol and progesterone. About 80-90% of circulating cortisol is bound to CBG. Albumin bound cortisol is reported to represent 14% and free cortisol 6% of total plasma cortisol under basal conditions. The CBG 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 CBG.

Defects in the gene encoding CBG are the cause of corticosteroid binding globulin deficiency (CBG deficiency), a rare disorder characterized by reduced CBG production that results in hypo/ hypertension and muscle fatigue. The plasma concentration of CBG 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, CBG is present at half of the normal adult level and prepubertal children have higher levels than adults. Plasma CBG levels increase during pregnancy and are decreased in cirrhosis. Estrogen therapy (e.g. oral hormonal contraception) or implantation during pregnancy cause a very marked increase of the CBG concentration. Decreased levels of CBG are observed in women with polycystic ovary syndrome, hypoproteinemia, Cushing's syndrome or corticoid treatment and some cases of vitamin B12 deficiency. Extremely low levels of CBG have been reported in patients with septic shock.

Measurement of corticosteroid binding globulin is important to the interpretation of cortisol levels. The concentration of unbound cortisol, which is biologically active, can be calculated from the concentration of total cortisol and that of CBG on the basis of mass action.

Areas of investigation:

Immune response, infection and inflammation

Liver disease

Steroid hormones

Reproduction

4. TEST PRINCIPLE

In the BioVendor Human Corticosteroid Binding Globulin ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human corticosteroid binding globulin antibody. After 60 minutes incubation and washing, biotin labelled monoclonal anti-human CBG antibody is added and incubated with the captured CBG for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of corticosteroid binding globulin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	50 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5 – 1 000 μl with disposable tips
- Multichannel pipette to deliver 100 μl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiterate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

- All reagents need to be brought to room temperature prior to use
 - Always prepare only the appropriate quantity of reagents for your test
 - Do not use components after the expiration date marked on their label
- Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Biotin Labelled Antibody

Streptavidin-HRP Conjugate

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

The resulting concentration of the CBG in the stock solution is **200 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	200 ng/ml
300 µl of stock	300 µl	100 ng/ml
300 µl of 100 ng/ml	300 µl	50 ng/ml
300 µl of 50 ng/ml	300 µl	25 ng/ml
300 µl of 25 ng/ml	300 µl	12.5 ng/ml
300 µl of 12.5 ng/ml	300 µl	6.25 ng/ml
300 µl of 6.25 ng/ml	300 µl	3.13 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted and/or diluted Standard solutions.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Stability and storage:

Do not store the reconstituted Quality Controls.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) 10-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x)+ 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures corticosteroid binding globulin in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C . Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples (serum, plasma) 1 000x with the Dilution Buffer just prior to the assay in two steps as follows:

Dilution A (50x):

Add 5 μl of sample into 245 μl of Dilution Buffer for singlets, or preferably 10 μl of sample into 490 μl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Dilution B (20x):

Add 15 μl of Dilution A into 285 μl of Dilution Buffer to prepare final dilution (1 000x). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20° , or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at $2-8^{\circ}\text{C}$, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of corticosteroid binding globulin.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at 25°C for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at 25°C for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at 25°C for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **15 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 – 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine CBG concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 200	QC HIGH	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 100	QC LOW	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 50	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 25	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 12.5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 6.25	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 3.13	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of corticosteroid binding globulin (ng/ml) in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 13.5 ng/ml (from standard curve) x 1 000 (dilution factor) = 13.5 µg/ml.

To convert concentrations of CBG in µg/ml to µmol/l, divide by 52.

Example of calculation:

The measured concentration of sample calculated from the standard curve and multiplied by dilution factor is 13.5 µg/ml. CBG level in µmol/l: $13.5/52 = 0.26$ µmol/l.

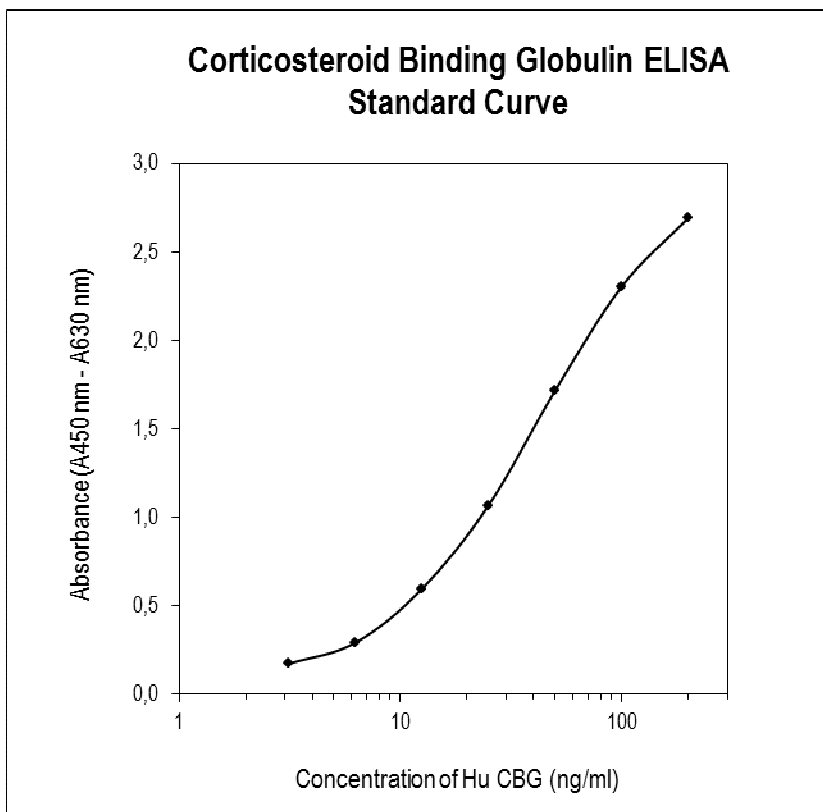


Figure 2: Typical Standard Curve for Human Corticosteroid Binding Globulin ELISA.

13. PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of BioVendor Corticosteroid Binding Globulin ELISA are presented in this chapter

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real CBG values in wells and is 0.01 ng/ml.

*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding CBG level of 200 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the CBG concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human corticosteroid binding globulin.

Sera of several mammalian species were measured in the assay. See results below.

For details please contact us at info@biovendor.com.

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

➤➤ Presented results are multiplied by respective dilution factor

• **Precision**

Intra-assay (Within-Run) (n=8)

Sample	Mean ($\mu\text{g/ml}$)	SD ($\mu\text{g/ml}$)	CV (%)
1	73.28	1.50	2.2
2	43.22	0.47	1.2

Inter assay (Run-to-Run) (n=5)

Sample	Mean ($\mu\text{g/ml}$)	SD ($\mu\text{g/ml}$)	CV (%)
1	34.70	2.35	6.8
2	39.59	2.88	7.3

• **Spiking Recovery**

Serum samples were spiked with different amounts of human CBG and assayed.

Sample	Observed ($\mu\text{g/ml}$)	Expected ($\mu\text{g/ml}$)	Recovery O/E (%)
1	30.38	-	-
	39.79	40.38	98.5
	53.87	50.38	106.9
	75.33	80.38	93.7
2	23.74	-	-
	32.48	33.74	96.3
	45.96	43.74	105.1
	74.51	73.74	101.0

• **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed ($\mu\text{g/ml}$)	Expected ($\mu\text{g/ml}$)	Recovery O/E (%)
1	-	28.02	-	-
	2x	14.36	14.01	102.5
	4x	6.72	7.00	95.9
	8x	3.55	3.50	101.5
2	-	68.53	-	-
	2x	32.99	34.26	96.3
	4x	16.35	17.13	95.4
	8x	8.50	8.57	99.3

- **Effect of sample matrix**

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals.

Results are shown below:

Volunteer No.	Serum ($\mu\text{g/ml}$)	Plasma ($\mu\text{g/ml}$)		
		EDTA	Citrate	Heparin
1	20.20	22.81	18.27	23.11
2	22.79	21.10	16.95	22.43
3	18.71	17.27	15.44	17.92
4	22.74	20.95	17.40	22.71
5	22.05	20.15	16.24	22.59
6	22.37	24.96	18.70	26.03
7	21.70	22.43	18.84	25.61
8	28.19	22.35	20.10	24.32
9	34.77	32.47	26.43	34.54
10	27.16	25.51	19.79	25.91
Mean (ng/ml)	24.04	23.00	18.82	24.52
Mean Plasma/Serum (%)		95.7	78.3	102.0
Coefficient of determination R²		0.97	0.97	0.97

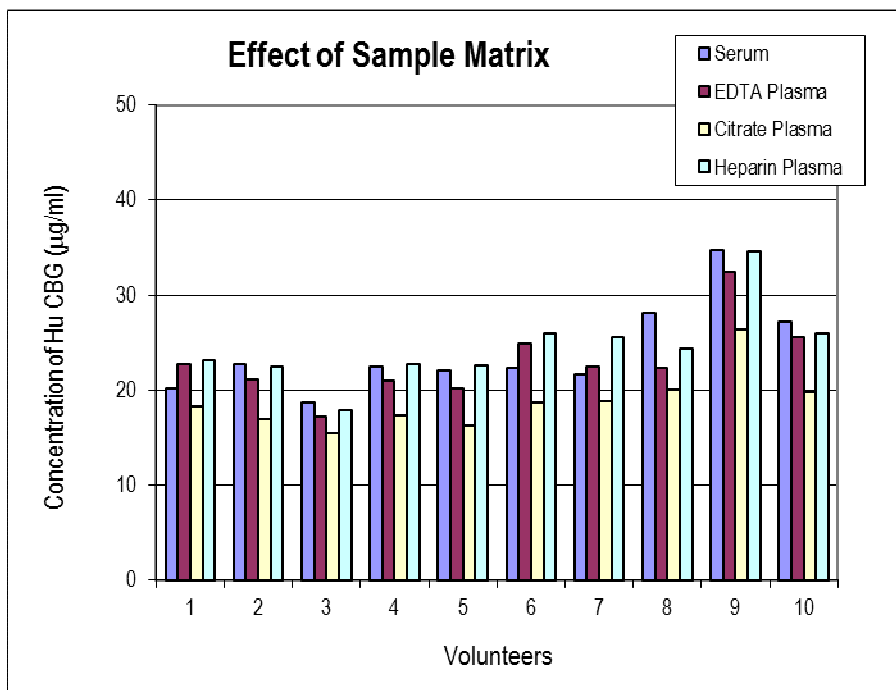


Figure 3: CBG levels measured using Human Corticosteroid Binding Globulin ELISA from 10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C, or preferably at -70°C. However, no decline in concentration of CBG was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum (µg/ml)	Plasma (µg/ml)		
			EDTA	Citrate	Heparin
1	-20°C	21.92	22.67	17.64	21.89
	2-8°C, 1 day	22.89	22.39	18.02	22.35
	2-8°C, 7 days	24.13	21.32	18.11	21.99
2	-20°C	18.70	21.85	16.83	20.64
	2-8°C, 1 day	23.59	21.83	16.26	20.83
	2-8°C, 7 days	19.47	21.34	16.25	19.21
3	-20°C	18.29	18.74	14.69	18.26
	2-8°C, 1 day	19.02	18.36	13.89	17.52
	2-8°C, 7 days	20.34	17.58	15.58	18.51

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human CBG in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (µg/ml)	Plasma (µg/ml)		
			EDTA	Citrate	Heparin
1	1x	30.96	30.89	26.14	32.36
	3x	31.23	28.70	25.90	33.95
	5x	29.17	30.43	25.32	31.38
2	1x	27.11	26.65	22.55	27.26
	3x	28.70	29.71	22.48	27.17
	5x	28.94	29.27	21.09	26.98
3	1x	23.62	24.12	21.34	25.50
	3x	26.27	25.29	20.12	31.45
	5x	25.44	24.85	19.90	25.17

- **Distribution of CBG in serum samples of healthy individuals and pregnant women**

Serum samples were taken from 120 normal, apparently healthy individuals and 86 pregnant women and measured in the assay. Results are shown below:

Samples	Mean (µg/ml)	Minimum value (µg/ml)	Maximum value (µg/ml)
Healthy individuals	40.16	20.01	102.22
Pregnant women	60.80	24.52	123.44

- **Reference range**

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for CBG levels with the assay.

14. DEFINITION OF THE STANDARD

The Standard used in this kit was prepared from human serum and fully corresponds to the previous formulation of the Standard which was native protein based (lots up to E12-042) and exhibited 100% steroid binding activity (as determined by saturation test with cortisol and progesterone).

Conversion of units

1 µg/ml = 0.0192 µmol/l

To convert concentrations of CBG in µg/ml to µmol/l, divide by 52. (Relative molecular mass of CBG is 52 kDa).

Example of calculation:

The measured concentration of sample calculated from the standard curve and multiplied by dilution factor is 13.5 µg/ml. CBG level in µmol/l: $13.5/52 = 0.26$ µmol/l.

15. METHOD COMPARISON

BioVendor Human Corticosteroid Binding Globulin ELISA has not been compared to any other immunoassay.

16. TROUBLESHOOTING AND FAQs

➤➤ Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

➤➤ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

➤➤ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

17. REFERENCES

»» References to corticosteroid binding globulin:







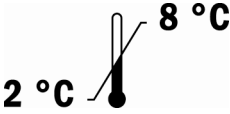

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»» References to this product

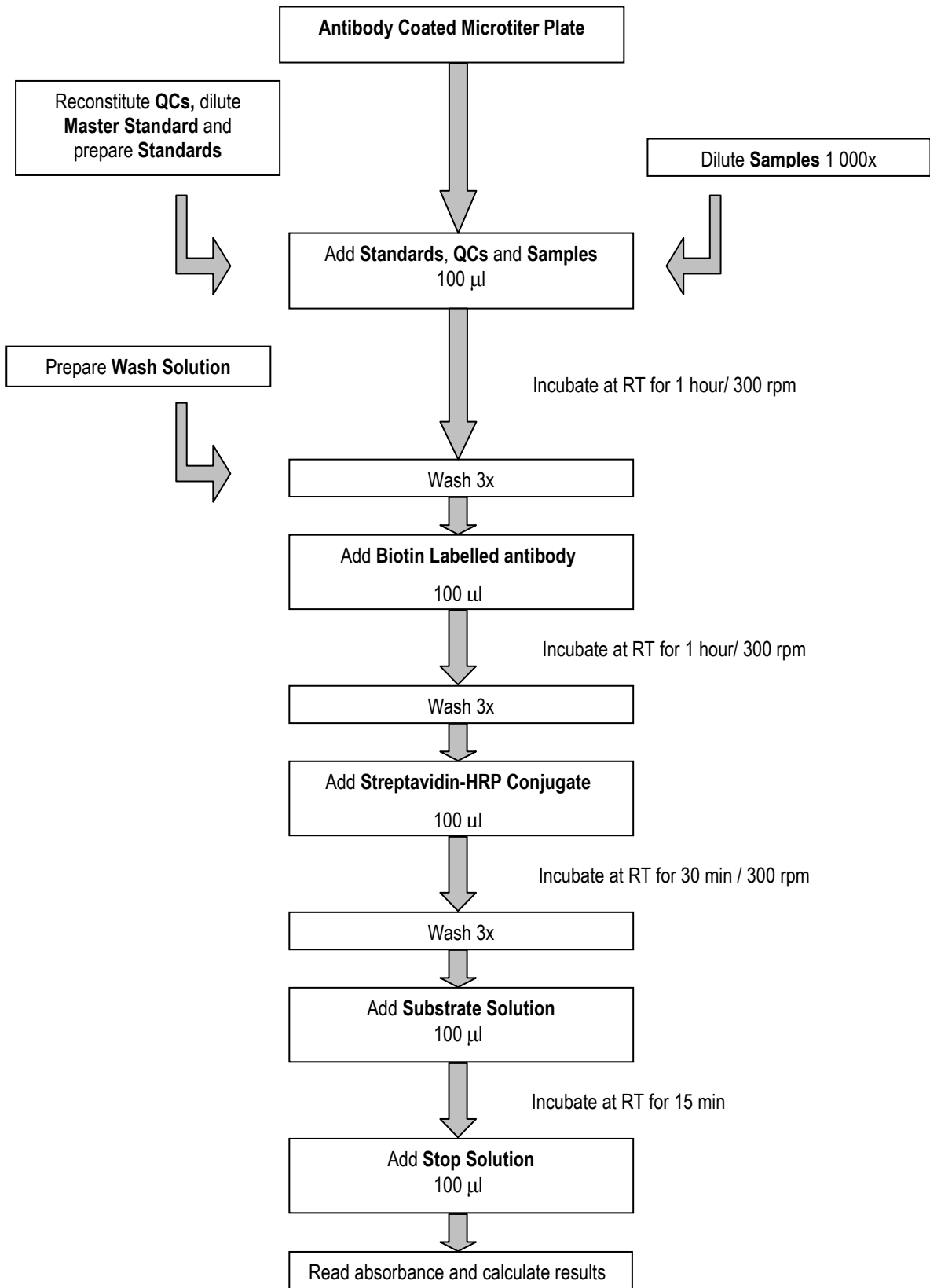
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»» For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

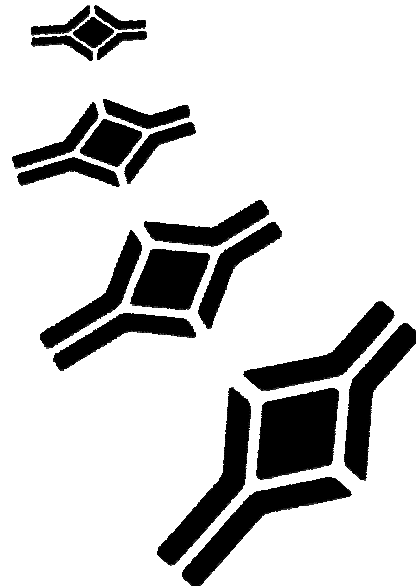


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