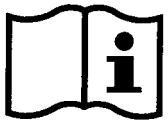
Product information



Useŕs Manual

SHBG ELISA

Enzyme immunoassay for the measurement of the sex-hormone-binding globulin (SHBG) in serum or plasma

REF **IB79131**

Σ **96**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

Manufactured For: Immuno-Biological Laboratories, Inc. (IBL-America)
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Please use only the valid version of the Instructions for Use provided with the kit.

Table of Contents

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1 INTRODUCTION

1.1 Intended Use

The **IBL-AMERICA SHBG ELISA** is an enzyme immunoassay for the measurement of the sex-hormone-binding globulin (SHBG) in serum or plasma (EDTA-, heparin- or citrate plasma).

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

2 PRINCIPLE OF THE TEST

The SHBG ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the SHBG molecule. During the first incubation, SHBG in the added sample binds to the immobilized antibody. The simultaneously added enzyme conjugate, which contains an anti-SHBG antibody conjugated to horseradish peroxidase, binds to the SHBG forming a sandwich complex. After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is proportional to the concentration of the analyte in the sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only. Not for use in diagnostic procedures. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents may contain Proclin, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-AMERICA.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-SHBG antibody (monoclonal).
2. **CAL 0 – 6 Standard (Standard 0 - 6),** 7 vials, 0.5 mL, ready to use;
   Concentrations: 0 – 4 – 16 – 32 - 65 – 130 - 260 nmol/L. The standards are calibrated against the following reference material: WHO International Standard for Sex Hormone Binding Globulin (08/266). Contain preservative.
3. **CONTROL low & high Control Low & High**, 2 vials, 0.5 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet. Contains preservative.
4. **BUF Assay Buffer,** 1 vial, 125 mL, ready to use, Contains preservative.
5. **ENZ CONJ Enzyme Conjugate,** 1 vial, 14 mL, ready to use, Anti-SHBG antibody conjugated with horseradish peroxidase; Contains preservative.
6. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
7. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.5 M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated), see ”Reagent Preparation“.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Tubes for dilution of standards, controls and samples
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 2 months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution
Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 1 week at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-AMERICA has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SAMPLE COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.
EDTA and citrate plasma samples may give slightly lower results.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Sample Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Sample receiving anticoagulants may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 4 days at 2 °C - 8 °C prior to assaying.
Samples held for a longer time (up to 2 months) should be frozen only once at -20 °C prior to assay.
Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard, the sample can be further diluted with *Assay Buffer* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10µL prediluted sample + 90µL Assay Buffer (mix thoroughly)
b) dilution 1:100: 10µL dilution a) 1:10 + 90 µL Assay Buffer (mix thoroughly)

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Predilution of standards, controls, and samples

Prior to the assay, all standards, controls and samples need to be diluted 1+100 in Assay Buffer.

Example: 10µL sample + 1000µL Assay Buffer

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
Take **50µL** of the prediluted standards, controls and samples for the SHBG ELISA

6.3 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each prediluted (1+100) ***Standard, Control*** and **sample** with new disposable tips into appropriate wells.
3. Incubate for **120 minutes** at room temperature.
4. Wash the wells as follows:
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times with 300 µL** diluted Wash Solution per well.
   If an automated plate washer is used:
   Rinse the wells **3 times with 400 µL** diluted Wash Solution per well.

   At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplet.!
5. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for **30 minutes** at room temperature.
7. Wash the wells as follows:
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times with 300 µL** diluted Wash Solution per well.
   If an automated plate washer is used:
   Rinse the wells **3 times with 400 µL** diluted Wash Solution per well.
   .
8. Add **100 µL** of ***Substrate Solution*** to each well.
9. Incubate for **15 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
11. Measure the optical density of the solution in each well at 450 nm filter (reading) and at 620 nm to 630 nm (background subtraction, recommended).
    It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 260 nmol/L. For the calculation of the concentrations this dilution factor has to be taken into account.

7 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-AMERICA directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 0.408 – 260 nmol/L.

8.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Substance	% Cross-reactivity
Corticoid binding globulin	< 0.2
Thyroxin binding globulin	< 0.04

8.3 Sensitivity

The analytical sensitivity of the IBL-AMERICA ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the *Standard 0* and was found to be 0.23 nmol/L.

The Limit of Blank (LoB) is 0.23 nmol/L.
The Limit of Detection (LoD) is 0.408 nmol/L.
The Limit of Quantification (LoQ) is 0.757 nmol/L.

8.4 Reproducibility

8.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (nmol/L)	CV (%)
1	10	41.67	2.3
2	10	66.75	4.6
3	10	87.37	3.2
4	10	133.62	4.8

8.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (nmol/L)	CV (%)
1	30	41.99	5.7
2	30	68.94	6.3
3	30	90.00	6.2
4	30	136.96	5.2

8.4.3 Inter Assay

The inter-assay (between-lots) variation was determined by repeated measurements of samples with 3 different kit lots

Sample	n	Mean (nmol/L)	CV (%)
1	18	44.04	8.1
2	18	61.32	8.9
3	18	92.27	11.7
4	18	160.92	8.2

8.5 Recovery

Recovery of the IBL-America ELISA was determined by adding increasing amounts of the analyte to different samples containing different amounts of endogenous analyte.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (nmol/L)		45.5	76.8	85.62	158.6
Average Recovery (%)		95.9	92.6	86.7	89.2
Range of Recovery (%)	from	92.9	88.3	85.5	87.4
	to	99.8	96.9	87.7	90.5

8.6 Linearity

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (nmol/L)		44.5	73.4	98.5	177.6
Average Recovery (%)		98.6	97.3	98.5	99.2
Range of Recovery (%)	from	96.1	93.8	94.2	96.2
	to	101.0	100.1	100.4	101.6

9 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

9.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of SHBG in a sample.

9.3 High-Dose-Hook Effect

Hook effect was not observed in this test up to a concentration of 11350 nmol/L of SHBG.

10 LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-AMERICA.

10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore