



SHBG ELISA



BL-40-E

IN VITRO DIAGNOSTIC USE

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1 INTRODUCTION

Sex-hormone-binding globulin (SHBG) is a β -globulin that specifically binds steroid hormones. The major site of SHBG synthesis is thought to be the hepatocytes. Its production is regulated by androgen/estrogen balance, thyroid hormones, insulin and dietary factors, among others. SHBG is involved in the transport of sex steroids in plasma. Its concentration is a major factor regulating their distribution between protein-bound and free states.

Determination of SHBG concentration is mainly of importance in the evaluation of mild disorders of androgen metabolism and it allows identification of women with hirsutism who are likely to respond to estrogen therapy.

Testosterone/SHBG –ratios correlate well with both measured and calculated values for free testosterone, and help to discriminate between subjects with excessive androgen activity and normal individuals.

2 PRINCIPLE OF THE TEST

A monoclonal antibody specific to SHBG is immobilized on microwell plates, and another monoclonal antibody, also specific to SHBG, is conjugated with horseradish peroxidase (HRP).





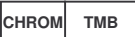
SHBG from the sample is bound to the plates. After a washing step, HRP conjugate is added. After a second washing step, enzyme substrate is added. The enzymatic reaction is proportional to the amount of SHBG in the sample. The reaction is terminated by adding stopping solution. Absorbance is measured on a plate reader.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- 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.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4 . It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.
The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

-  12x8 (break apart) strips
96 wells
Wells coated with mouse monoclonal SHBG antibody
-  N=0 to 4
5 vials, 0.5 ml
See exact values on the vial labels
Ready to use
-  Assay buffer
1 vial, 80 ml
Ready to use
-  Enzyme Conjugate, 100 x conc.
1 vial, 0.2 ml,
Mouse monoclonal SHBG antibody conjugated with horseradish peroxidase.
See "Preparation of Reagents"
-  1 vial, 12 ml
Ready to use
TMB

6.

STOP	SOLN
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 1 vial, 12 ml
Ready to use
Contains 0.5M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.
7.

WASH	SOLN	CONC
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 40 x conc.
1 vial, 25 ml
8.

CONTROL	N
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 N=1, 1 vial, 0.5 ml
Ready to use
Please find the exact value at the vial label. (Range ± 15%)

Note: Additional Zero Calibrator for Sample dilution available on request.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Aqua dest.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foil bag has been open care should be taken to close it tightly again.

4.4 Preparation of Reagents

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

Calibrators, Control Serum

Dilute Calibrators, Control Serum 1:21 with Assay Buffer, e.g. Dilute 10 µl calibrators, controls or samples with 200 µl Assay Buffer

Enzyme Conjugate Solution

Dilute the Conjugate Concentrate 1:101 with Assay Buffer as follows:

No. of strips	Concentrated Conjugate	Assay Buffer
1	10 µl	1 ml
2	20 µl	2 ml
3	30 µl	3 ml
4	40 µl	4 ml
5	50 µl	5 ml
6	60 µl	6 ml
7	70 µl	7 ml
8	80 µl	8 ml
9	90 µl	9 ml
10	100 µl	10 ml
11	110 µl	11 ml
12	120 µl	12 ml

This Solution is stable at 2-8°C for 8 weeks.

Wash Solution

Dilute 25 ml of concentrated Wash Solution with 975 ml aqua dest. to a final volume of 1000 ml. The diluted Wash Solution is stable for 8 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line have to be informed written, 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 SPECIMEN

5.1 Specimen collection

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Serum and heparin plasma can be used. EDTA-plasma may give slightly lower results. No interferences resulting from hemolysis, lipemia or bilirubin have been observed.

5.2 Specimen storage

Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimen held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen dilution

Dilute all samples 1:21 with Assay Buffer, e.g. Dilute 10 µl of all samples with 200 µl Assay Buffer.

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens 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 pipet tips for each calibrator, control of sample in order to avoid crosscontamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be 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. Therefore, if the Optical Density is too high or too low, the substrate incubation time can be decreased or increased, respectively.

6.2 Procedural Notes

- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- The concentration of the samples can be read directly from this calibrator curve.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dilute calibrators, Control Serum and samples 1:20 with Assay Buffer, e.g. Dilute 10 µl calibrators, controls or samples with 200 µl Assay Buffer. Dilute Conjugate 1:100 with Assay buffer as described before.
3. Pipette **100 µl** of Assay Buffer into each well.
4. Dispense **25 µl** of diluted Calibrators, controls and samples **with new disposable tips** into appropriate wells.
5. Cover the plate and incubate for **30 minutes** at room temperature.
6. Briskly shake out the contents of the wells.
Rinse the wells 3 times with diluted Wash Solution. Strike the wells sharply on absorbent paper to remove residual water droplets.
Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Dispense **100 µl** diluted Enzyme Conjugate (see „Preparation of Reagents“) into each well.
8. Cover the plate and incubate for **15 minutes** at room temperature.
9. Briskly shake out the contents of the wells.
Rinse the wells 3 times with diluted Wash Solution. Strike the wells sharply on absorbent paper to remove residual water droplets.
10. Add **100 µl** of TMB Substrate Solution to each well.
11. Cover the plate and incubate for **12 minutes** at room temperature (20-25°C) or for **8 minutes** at room temperature (26°C and more).
12. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
13. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in nmol/l 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 of SHBG in nmol/l from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data deduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with SHBG concentration higher than that of the highest calibrator have to be diluted with zero calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

7 Assay Characteristics

7.1 Expected values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Serum samples from apparently healthy women and men were assayed using the SHBG ELISA, with the following results:

	Number of Samples	SHBG NMOL/L	
		Mean	Range
Men	102	43	15 - 100
Women	44	62	15 - 120

7.2 Specificity

Specificity of the SHBG ELISA was studied by measuring apparent SHBG response caused by high levels of TBG (Thyroxine Binding Globulin) and CBG (Cortisol Binding Globulin). No cross-reactions were found when testing up to 500 mg/l of TBG and 500 mg/l of CBG.

7.3

7.4 Sensitivity

The minimum detectable concentration of SHBG by this assay is estimated to be 0.2 nmol.

7.5 Accuracy

Quality Control

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 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 patient 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.

7.6 Precision

7.6.1 Intra Assay Variation

The within assay variability is shown below:

Patient	Number of Replicates	Mean nmol/l	SD nmol/l	CV %
1	16	4.5	0.39	8.6
2	16	16	0.68	4.3
3	16	57	1.7	3.0
4	16	158	8.4	5.3

7.6.2 Inter Assay Variation

The between assay variability is shown below:

Patient	Number of Replicates	Mean nmol/l	SD nmol/l	CV %
1	16	3.8	0.44	11.6
2	16	19	1.6	8.4
3	16	63	5.5	8.7
4	16	194	14	7.2

7.7 Recovery

A known amount of SHBG was added to three patient sera and the quantities recovered were measured. The results are shown in the following table:

Sample	Endogenous SHBG nmol/l	Added SHBG nmol/l	Expected SHBG nmol/l	Observed SHBG nmol/l	Recovery %
1	39	6.5	45.5	42	92
1	39	28.5	67.5	67	99
1	39	165	204	208	102
2	61	6.5	67.5	63	93
2	61	28.5	89.5	91	102
2	61	165	226	224	99
3	157	6.5	163.5	170	104
3	157	28.5	185.5	210	113
3	157	165	322	307	95

8 LIMITATIONS OF USE

8.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen - Collection'.

8.2 High-Dose-Hook Effect

No hook effect was observed in this test up to 10000 nmol/l of SHBG.

9 LEGAL ASPECTS

9.1 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.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 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.

Claims submitted due to customer misinterpretation of laboratory results subject to point 9.2. are also invalid. 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.

10 REFERENCES

1. Moore, J.W. and Bulbrook R.D. (1988) *The epidemiology and function of sex hormone binding globulin*. IN: Oxford Reviews of Reproductive Biology, 10: 180-236.
2. Selby, C. (1980) *Sex hormone binding globulin: origin, function and clinical significance*. Ann Clin Biochem 27: 532-541