SHBG (Human) ELISA Kit

Catalog Number KA1913

96 assays

Version: 05

Intended for research use only

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Introduction

Intended Use

The SHBG (Human) ELISA Kit is an enzyme immunoassay for the quantitative measurement of SHBG in serum and heparin plasma.

Background

Sex-hormone- binding globulin (SHBG) is a β-globulin that specifically binds steroid hormones. Its molecular weight is 86 kDa/mol. 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 response 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.

Principle of the Assay

The SHBG (Human) ELISA Kit 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. An aliquot of sample containing endogenous SHBG is incubated in the coated well. After a washing step, enzyme conjugate is added, which is a monoclonal anti-SHBG antibody conjugate with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of SHBG in the sample
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of SHBG in sample.

General Information

Materials Supplied

List of component

Component	Amount
Microtiterwells: Wells coated with anti-SHBG antibody (monoclonal).	96 (8 x 12) wells
Standard A (0 nmol/L), contains preservative, ready to use.	0.5 mL
Standard B (4 nmol/L), contains preservative, ready to use.	0.5 mL
Standard C (16 nmol/L), contains preservative, ready to use.	0.5 mL
Standard D (65 nmol/L), contains preservative, ready to use.	0.5 mL
Standard E (260 nmol/L), contains preservative, ready to use.	0.5 mL
Control, contains preservative, ready to use. For control values and ranges please refer to vial label or QC-Datasheet.	0.5 mL
Assay Buffer, contains preservative, ready to use.	80 mL
Enzyme Conjugate, ready to use, anti-SHBG antibody conjugate to horseradish peroxidase, contains preservative.	14 mL
Substrate Solution, ready to use, tetramethylbenzidine (TMB).	14 mL
Stop Solution, ready to use, contains 0.5 M H_2SO_4. Avoid contact with the stop solution. It may cause skin irritation and burns.	14 mL
Wash Solution, 40X concentrated solution.	25 mL

Note: The standards are calibrated against human SHBG, WHO standard (NIBSC 08/266).

Storage Instruction

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 two months if stored as describe above.

Materials Required but Not Supplied

- ✓ A microtiter plate calibrated reader (450 ± 10 nm)
- ✓ Calibrated variable precision micropipettes
- ✓ Absorbent paper
- ✓ Distilled or deionized water
- ✓ Tubes for sample/standard dilution
- ✓ Timer
- ✓ Graph paper or software for data reduction

Precautions for Use

- Warnings and Procedural Cautions

1. This kit is for research use only. For professional use only.
2. All reagents of this kit which contain human serum or plasma have been tested an 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 stating 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 reagents 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 the 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 specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in area where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens 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 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 results slightly different.
17. Avoid contact with Stop Solution containing of 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 eye 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 the manufacturer.

- Limitations of Use

✓ Reliable and reproducible results will be obtained when the assay procedure is performed with complete understanding of the package insert instruction and with adherence to good laboratory practice.

✓ Any improper handling of smaples or modification of this test might influence the results.

- Interfering Substances

✓ Haemoglobin, bilirubin and triglyceride have no influence on the assay results.

- 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.

- Damaged Test Kits

  In case of any severe damage to the test kit or components, the manufacturer 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.

- Reliability or 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 standard 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 the manufacturer.

Assay Protocol

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.
  Diluted 25 mL of concentrated Wash Solution with 975 mL deionized water to a final volume of 1000 mL.
  The diluted Wash Solution is stable for 2 weeks at room temperature.

Sample Preparation

Serum and heparin plasma can be used in this assay.
EDTA-plasma may give slightly lower results.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

- Specimen Collection

✓ Serum
  Collect blood by veinpuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Individuals receiving anticoagulant treatment may require increased clotting time.

✓ Plasma
  Whole blood should be collected in to centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.

- Specimen Storage and Preparation
  Specimens should be capped and may be stored for up to 2 days at 2°C to 8°C prior to assaying.
  Specimens 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.

- Specimen Dilution
  Prior to the assay each sample needs to be diluted 1:20 in assay Buffer.
  For details please see step in Assay Procedure section.
  If in a initial assay, as specimen is found to contain more than the highest standard, the specimens can be further diluted with Assay Buffer and reassayed as described in Assay Procedure.
  For the calculation of the concentration this dilution factor has to be taken into account.

Assay Procedure

- All reagents and specimens must be allowed to come to room temperature before use. All reagents muct 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 in linearly proportional to time and temperature.
- Pipetting of samples should not exceed 10 minutes to avoid assay drift. If more than one plate is used in the same run, it is recommended to include a standard curve in each plate.
- Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dilute each Standard, Control and sample 1:20 with Assay Buffer in a separated non-adsorptive 96 well plate. (1 part Standard/Control/Sample +19 part Assay Buffer)
   Example: 10 µL Standard + 190 µL Assay Buffer
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
3. Dispense 100 µL Assay Buffer in to the required wells of the coated microtiter wells.
4. Dispense 25 µL of each diluted Standard, Control, and sample with new disposable tips into appropriate wells. Thoroughly mix for 5 seconds. It is important to have a complete mixing in this step.
5. 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 (300 – 400 µL per well). Strike the wells sharply on absorbent paper to remove residual 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 Enzyme Conjugate in each well.
8. 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 (300 – 400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
10. Add 100 µL of Substrate Solution to each well.
11. Incubate for 12 minutes at room temperature (20°C - 25°C), 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. Determined the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.
    It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Data Analysis

Calculation of Results

- Calculate the average absorbance values for each set of standards, controls and smaples.
- Using semi-logarithmic 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.
- Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
- Automated method: The results in the IFU have been calculated automatically using 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
- 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.
- Example of Typical Standard Curve

  The following data is for demonstration only and cannot be used in placed of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard A (0 nmol/L)	0.01
Standard B (4 nmol/L)	0.08
Standard C (16 nmol/L)	0.30
Standard D (65 nmol/L)	1.07
Standard E (260 nmol/L)	2.04

- Expected Normal Values

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

  In a study conducted with apparently normal healthy adults, using the SHBG ELISA the following values are observed:

Population	N	SHBG nmol/L	
		Mean	Range
Males	102	43	15-100
Females	44	62	15-120

The results alone should not be the only reason for any therapeutic consequences. The result should be correlated to other clinical observations and detection tests.

- 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 abnormal 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 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 analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials individual 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 the manufacturer directly.

Performance Characteristics

- Assay Dynamic Range

  The range of the assay is between 0.77 – 260 nmol/L.

- Specificity (Cross-Reactivity)

  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.

- Sensitivity

  The analytical sensitivity of the SHBG ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Standard A (S0) and was found to be 0.77 nmol/L.

- Reproducibility

✓ Intra-assay precision

The within assay variability is show below:

Sample	n	Mean (nmol/L)	CV (%)
1	16	10.3	9.0
2	16	44.0	5.4
3	16	76.1	4.0
4	16	109.6	5.3

✓ Inter-assay precision

The between assay variability is show below:

Sample	n	Mean (nmol/L)	CV (%)
1	16	9.8	8.0
2	16	44.9	3.0
3	16	73.4	5.3
4	16	106.8	3.1

✓ Recovery

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

Sample	Endogenous SHBG nmol/L	Added SHBG (Expected Value) nmol/L	Measured SHBG (total) nmol/L	Measured value minus endogenous value (observed value) nmol/L	Recovery %
1	8.2	32	39.0	30.8	96
	8.2	16	23.1	14.9	93
2	10.8	32	39.0	28.8	90
	10.8	16	26.7	15.9	99
3	11.3	32	37.4	26.1	82
	11.3	16	25.2	13.9	87

✓ Linearity

Three sample serum samples were diluted with assay Buffer to 1:2, 1:4 and 1:8. SHBG- values were assayed, and the results were corrected using dilution factors.

Recovery results of these dilution testes are shown in the following table.

Sample	Undiluted SHBG nmol/L	Recovery %		
		At dilution 1:2	At dilution 1:4	At dilution 1:8
1	89	101	92	110
2	99	97	96	91
3	177	99	86	81

Resources

References

1. Moore, J. W. and Bulbrook R. D. (1988). The epidemiology and function of sex hormone binding globulin. IN Oxford Rewievs of Reproductive Biology, 10: 180 - 236.
2. Selby, C. (1990). Sex hormone binding globulin: origin, function and clinical significance. Ann. Clin. Biochem. 27: 532 - 541.

Plate Layout

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