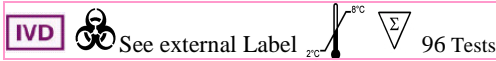


**AccuDiag™  
 SHBG  
 ELISA Kit**

**Cat# 2043-6**



<b>Test</b>	<b>Sex-hormone-binding globulin</b>
<b>Method</b>	<b>Enzyme Linked Immunosorbent Assay</b>
<b>Principle</b>	<b>Sandwich Complex</b>
<b>Detection Range</b>	<b>0.77-260 nmol/L</b>
<b>Sample</b>	<b>10 µL serum / plasma</b>
<b>Total Time</b>	<b>~60 min.</b>
<b>Shelf Life</b>	<b>12-14 Months from the manufacturing date</b>
<b>Specificity</b>	<b>100%</b>
<b>Sensitivity</b>	<b>0.77 nmol/L</b>

**INTENDED USE**

The Diagnostic Automation, Inc. (DAI) SHBG ELISA is an enzyme immunoassay for measurement of SHBG in serum and heparin plasma.

**SUMMARY AND EXPLANATION**

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 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.

**TEST PRINCIPLE**

The DAI SHBG 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 conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration SHBG in the sample.

Having added the substrate solution, the intensity of color developed is proportional to the concentration of SHBG in the sample.

**SPECIMEN COLLECTION AND PREPARATION**

- Serum or 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.

**1. Specimen 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. Patients receiving anticoagulant therapy may require increased clotting time.

**Plasma:**

Whole blood should be collected into centrifuge tubes containing anti coagulant (eg. Sarstedt Monovette with the appropriate plasma preparation) 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.

**3. Specimen Dilution**

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be 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:**

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

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**

- Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-SHBG antibody (monoclonal).
- Standard (Standard 0-4)**, 5 vials, 0.5 mL, ready to use; Concentrations: 0 – 4 – 16 – 65 – 260 nmol/L The standards are calibrated against human SHBG, WHO Standard (NIBSC 08/266) Contain preservative.
- Control**, 1 vial, 0.5 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet. Contains preservative.
- Assay Buffer**, 1 vial, 80 mL, ready to use. Contains preservative.
- Enzyme Conjugate**, 1 vial, 14 mL ready to use, Anti-SHBG antibody conjugated to horseradish peroxidase; Contains preservative.
- Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
- Stop Solution**, 1 vial, 14 mL, ready to use, Contains 0.5M H<sub>2</sub>SO<sub>4</sub>.
- Avoid contact with the stop solution. It may cause skin irritations and burns.
- Wash Solution**, 1 Vial, 25 mL (40X Concentrated) See " Preparation of Reagents"

**Materials required but not provided**

- A microtiter plate calibrated reader (450 ± 10 nm).

2. Calibrated variable precision micropipettes.
3. Absorbent paper.
4. Distilled or Deionized water.
5. Timer Semi logarithmic graph paper or software for data reduction.

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 **100µL** of **Stop Solution** to each well.
13. Determine 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.

## 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 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.*

### 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 Material Safety Data Sheet.

### Damaged Test Kits

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

## TEST PROCEDURE

### 1. General Remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. 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.
5. As a general rule the enzymatic reaction is 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 on each plate. Each run must include a standard curve.

### 2. Test Procedure

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* (1 part Standards/Control/sample + 19 parts *Assay Buffer*)  
*Example: 10µL Standard + 190 µL Assay Buffer*
3. Dispense **100 µL Assay Buffer** into each well.
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** into 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.

## RESULTS

1. Calculate the average absorbance values for each of standards, controls and samples.
2. 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.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter logistics is the preferred method. 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.

### Example of Typical Standard curve

The following data is for demonstration only and **cannot** be used in place of data generation s at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 nmol/L)	0.01
Standard 1 (4 nmol/L)	0.08
Standard 2 (16 nmol/L)	0.30
Standard 3 (65 nmol/L)	1.07
Standard 4 (260 nmol/L)	2.04

## EXPECTED NORMAL VALUES

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

In a study conducted with apparently normal healthy adults, using the DAI 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 results should be correlated to other clinical observations and diagnostic 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 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 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.

After checking the above mentioned items without finding any error contact DAI directly.

## PERFORMANCE CHARACTERISTICS

### Assay Dynamic Range

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

### Specificity of Antibodies (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 DAI ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be 0.77 nmol/L.

### Reproducibility

#### Intra Assay

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

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 patient sera and the quantities recovered were measured. The results are shown in the following table.

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

### Linearity

Three patient 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 tests are shown in the following table:

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

### LIMITATIONS OF PROCEDURE

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.

#### Interfering Substances

Hemoglobin, bilirubin and triglycerides have no influence on the assay results.

#### Drug Interference

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



#### High-Dose Hook Effect

No hook effect was observed in this test up to 40,000 nmol/L of SHBG.

### PRECAUTIONS

1. This kit is for research use only. 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 1, 2, 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 (21-26°C) before starting the test. Temperature will affect the absorbance reading of the assay. However, values for the samples will not be affected.
10. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas 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 the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate 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<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation 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 Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DAI.

 <b>ISO 13485</b> <b>ISO 9001</b>	
 <b>Diagnostic Automation/Cortez Diagnostics, Inc.</b> <b>23961 Craftsman Road, Suite E/F,</b> <b>Calabasas, California 91302 USA</b>	
<b>Date Adopted</b>	<b>Cat # 2043-6</b>
<b>2013-04-01</b>	<b>AccuDiag™- SHBG</b> <b>ELISA -2013</b>
<b>Revision B Date: 11-13-2013</b>	

## STORAGE

1. When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
2. Opened reagents must be store at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C.
3. Once the foil bag has opened kits retain activity for two months if stored as described above.

## 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.