AccuDiag™ SHBG ELISA Kit

REF 2043-6

IVD 2°C 8°C 96 Tests

INTENDED USE

The DAI SHBG ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of the sex- hormone-binding globulin (SHBG) in serum or plasma (EDTA-, heparin- or citrate plasma)

SUMMARY AND EXPLANATION

Sex-hormone-binding globulin (SHBG), a homodimeric glycoprotein of 95 kD, is synthesized in the liver and has a half- values time of 7 days in plasma. SHBG specifically binds steroid hormones with high affinity (DHT > testosterone > estrone/estradiol > DHEA/ androstenedione/ estriol), and its main function is sex-steroid transport within the blood stream and to extravascular target tissues. SHBG also plays a key role in regulating bioavailable sex-steroid concentrations through competition of sex steroids for available binding sites and fluctuations in SHBG concentrations. SHBG concentration in blood shows high inter-individual variability and is influenced by androgen/estrogen balance, nutritional status, body mass index, sex, insulin concentration among others. SHBG levels in pre-pubertal children are higher than in adults. Men have lower levels compared to women.

SHBG levels are increased in older men, during pregnancy, hormone replacement therapy, liver cirrhosis, hyperthyroidism, hypogonadism, androgenization in women, and after intake of contraceptives or anti-epileptics. SHBG levels are decreased in obesity, polycystic ovary syndrome (PCOS), Cushing Syndrome, hypothyroidism and after glucocorticoid therapy.

In postmenopausal women, SHBG may also predict the future development of type 2 diabetes mellitus. Moreover, the free testosterone index (FTI) can help to identify women with androgenization

(FTI (%) = (total testosterone / SHBG) × 100).

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

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma(EDTA-, heparin- or citrate plasma) can be used in this assay.

- EDTA-and citrate plasma may give slightly lower results.
- Please note: Samples containing sodium azide should not be used in the assay.

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 (e.g. 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 4 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time(upto 3 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution
If in an initial assay, a 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 concentrations this dilution factor has to be taken into account.
Example:

a) *Dilution 1:10: (mix thoroughly)* — *10 µL prediluted sample + 90 µL Assay Buffer*

b) *Dilution 1:100: thoroughly).* — *10 µL dilution a) 1:10 + 90 µL Assay Buffer (mix*

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
Wells coated with anti-SHBG antibody (monoclonal).

2. ***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 human SHBG, WHO Standard (NIBSC 08/266)
Contain preservative.

3. ***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. ***Assay Buffer***, 1 vial, 125 mL, ready to use,
Contains preservative.

5. ***Enzyme Conjugate***, 1 vial, 14 mL, ready to use,
Anti-SHBG antibody conjugated to horseradish peroxidase;
Contains preservative.

6. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
Tetramethylbenzidine (TMB).

7. ***Stop Solution***, 1 vial, 14 mL, ready to use, contains 0.5 M H2SO4,

Avoid contact with the stop solution. It may cause skin irritations and burns.

8. ***Wash Solution***, 1 vial, 30 mL (40X concentrated), see "Preparation of Reagents".

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 sample / standard dilution
- Timer
- Graph paper or software for data reduction

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 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 Safety Data Sheet.

Damaged Test Kits
In case of any severe damage to the test kit or components, DACD 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

- 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 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.

2 Predilution of standards, controls and samples
Prior to the assay, all standards, controls and patient samples need to be diluted 1+100 in Assay Buffer
Example: 10 µL sample + 1000 µL Assay Buffer
Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
Take **50 µL** of the prediluted standards, controls and samples for the SHBG ELISA

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 µLof each prediluted *Standard*, *Control* and sample with new disposable tips into appropriate wells

3. Incubate for **120 minutes** at room temperature
4. 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!
5. Dispense **100 µL *Enzyme Conjugate*** into each well.
6. Incubate for **30 minutes** at room temperature.
7. 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.
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. 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*.

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	0.02
Standard 1	4 nmol/L	0.09
Standard 2	16 nmol/L	0.27
Standard 3	32 nmol/L	0.49
Standard 4	65 nmol/L	0.84
Standard 5	130 nmol/L	1.36
Standard 6	260 nmol/L	1.93

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	Mean (nmol/L)	Median (nmol/L)	2.5th - 97.5th Percentile (nmol/L)	Range (min. - max.) (nmol/L)
Males	78	45.3	42.1	17.7 - 92.8	16.8 - 113.2
Females	40	65.0	58.2	20.4 - 126.7	16.1 - 128.4

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 your distributor or DACD directly.

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range
The range of the assay is between 0.408-260 nmol/L.

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

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 0.23 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.

Reproducibility
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

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

Interlot
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

Recovery
Recovery of the DAI ELISA was determined by adding increasing amounts of the analyte to different patient 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

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
fro	96.1	93.8	94.2	96.2

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Range of Recovery (%)	m				
	to	101.0	100.1	100.4	101.6

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 (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.

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

PRECAUTIONS

1. This kit is for in vitro diagnostic 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 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 (18 °C to 25 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient 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 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 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 DACD.

STORAGE

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 described above.

LEGAL ASPECTS

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 DACD
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Therapeutic Consequences
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated previously under "Reliability of Results". 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 therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

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 "Therapeutic Consequences" 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.

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