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**IVD**



See external label



2°C-8°C



Σ=96 tests

**REF**

Cat # 2043-6

# SHBG

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## Clinical background

Sex-hormone-binding globulin (SHBG) is a B-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.

## PRINCIPLE OF 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.

## MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipette with disposable plastic tips; 25µl (standards, samples)
- Multichannel pipette with disposable plastic tips
- Lid or sealing tape for microwell plate
- Reagent troughs
- Aspiration device
- Photometer (plate or strip reader) 450 nm

## PRECAUTIONS

The SHBG ELISA TEST is intended for in vitro use only.

The reagents contain the preservative thimerosal. The Control Serum has been prepared from human sera shown to be negative for HbsAg, HIV antibodies and HCV antibody. Nevertheless such tests are unable to prove the complete absence of viruses and the Control Serum should therefore be handled taking appropriate precautions.

## SPECIMEN COLLECTION AND HANDLING

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. Specimens may be stored at 2-8°C for brief periods (approximately two days). For longer storage, specimens should be frozen. Frozen specimens should be well mixed after thawing and before assay. Avoid repeated freezing and thawing.

### Dilution of Samples

Serum samples with SHBG concentrations greater than the highest standard should be diluted further with the Assay Buffer. Correct the result using an appropriate dilution factor.

(See chapter 7.1 “Preparation of Reagents and Samples”)

## CONTENTS OF KIT

Reagents are sufficient for 96 wells. The kit should be stored at 2 - 8 °C.

The unopened kit is stable until the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the component.

1. **Breakapart Wells.** 96 wells on a plate coated with mouse monoclonal SHBG antibody, packed in a laminate bag. Ready for use.
2. **Assay Buffer,** 1 x 80 ml. Ready for use.
3. **SHBG Standards.** Standards A – E, 0.5 ml. Calibrated against human SHBG, WHO Standard (NIBSC 95/560). The standard values are about 0, 4, 16, 65 and 260 nmol/l. Exact standard values are given on the label of each vial. (See chapter 7.1 “Preparation of Reagents and Samples”)
4. **Control,** 0.5 ml. (See chapter 7.1 “Preparation of Reagents and Samples”)
5. **Enzyme Conjugate,** 14 ml. Ready for use. Mouse monoclonal SHBG antibody conjugated with horseradish peroxidase.
6. **Wash Solution,** 25 ml, (40x conc.). (See chapter 7.1 “Preparation of Reagents and Samples”)
7. **TMB Substrate Solution,** 14 ml. TMB. Ready for use.
8. **Stop Solution,** 14 ml. Ready for use. Avoid contact with eyes and skin.

## TEST PROCEDURE

### Preparation of Reagents and Samples

#### 1. Standards, controls, samples

Dilute standards, controls and samples 1:20 with Assay Buffer (1 part standards/control/sample + 19 parts Assay Buffer)

*Example:* 10 µL Standard + 190 µL Assay Buffer

## 2. Wash Solution

Dilute the 40x concentrate in 975 ml distilled water.

### Assay Procedure

Allow all reagents to reach room temperature before use.

1. Mark the wells to be used on the plate.
2. Pipet 100  $\mu$ l of Assay Buffer into each well.
3. Pipet 25  $\mu$ l of diluted standards, control and serum samples into appropriate wells and shake the plate for five seconds. (See chapter 7.1 *Preparation of Reagents and Samples*.)
4. Cover the plate and incubate for 30 minutes at room temperature.
5. Aspirate and wash the wells 3 times with 300  $\mu$ l of washing solution.
6. Pipet 100  $\mu$ l of Enzyme Conjugate into the wells.
7. Cover the plate and incubate for 15 minutes at room temperature.
8. Wash the wells as above (3 x 300 $\mu$ l).
9. At timed intervals add 100  $\mu$ l of TMB Substrate Solution into each well.
10. Cover the plate and incubate  
for 12 minutes at room temperature (20-25°C)  
for 8 minutes at room temperature (26°C and more)
11. Stop the reaction by adding 100  $\mu$ l of Stop Solution into each well at the same timed intervals as in step 9. Shake the plate gently to mix the solutions.
12. Measure the absorbance at 450 nm using a plate or strip reader at least 5 min after stopping the Substrate reaction.

### Notes on Technique

1. Protect the plates from draught, strong light or direct sunlight during the test procedure.
2. Careful aspiration of the washing solution is essential for good assay precision.
3. Since timing of the incubation steps is important to performance of the assay, pipette the samples and the conjugate without interruption. 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.
4. Adding of the TMB Substrate Solution starts a kinetic reaction that is terminated by dispensing the Stopping Solution. Keep the incubation times for each well the same by adding reagents at timed intervals.
5. Protected from light, absorbance values are stable for 60 minutes
6. Plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

## TEST PROCEDURE – SUMMARY

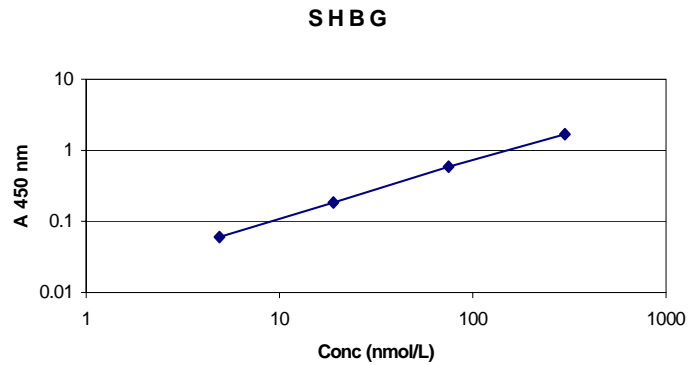
	Standards 0 – 260 nmol/l(1:20)	Control Serum (1:20)	Samples (1:20)
Mark the strips			
Pipet Assay Buffer (µl)	100	100	100
Pipet diluted standards, Control Serum and samples (µl)	25	25	25
Incubate for 30 minutes at room temperature			
Wash 3 times			
Pipet Enzyme Conjugate (µl)	100	100	100
Incubate for 15 minutes at room temperature			
Wash 3 times			
Pipet TMB Substrate Solution (µl)	100	100	100
Incubate for 15 minutes at room temperature			
Stop sol. (µl)	100	100	100
Mix			
Measure absorbance at 450 nm			

## RESULTS

1. Calculate the mean absorbance for each duplicate.
2. Subtract the absorbance value of the zero standard from the mean absorbance values of standards, control and samples.
3. Draw the standard curve on log-log graph paper by plotting absorbance values of standards against appropriate SHBG concentrations.
4. Read off the SHBG concentrations for the control and the samples.

Example of worksheet and standard curve of typical assay: **Not to be used** for calculation of actual test results. (EXAMPLE!).

Wells	Identity	A 450 nm	Conc. nmol/l
1-2	St 0 nmol/l	0.041	
3-4	St 4	0.096	0.055
5-6	St 20	0.236	0.195
7-8	St 75	0.621	0.580
9-10	St 300	1.731	1.690
11-12	Sample 1	0.203	0.162
13-14	Sample 2 (control)	0.461	0.420
15-16	Sample 3	1.153	1.102



## QUALITY CONTROL

It is recommended that internal controls be used in every assay. Control results should be within established ranges.

## EXPECTED VALUES

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

	Number of samples	SHBG nmol/l	
		Mean	range
Men	102	43	15-100
women	44	62	15-120

Each laboratory should determine its own reference range.

## Performance Characteristics

### Detection limit

On the basis of results of 16 replicate determinations of the zero standard, the minimum SHBG concentration detectable by the method is 0.2 nmol/l. The detection limit is defined as the value deviating by 2 SD from that of the zero standard.

### Precision

Intra- and inter-assay precisions were established by analyzing three patient sera of different SHBG concentrations. The results are shown in Tables 1 and 2.

**Table 1. Intra-assay precision**

Patients	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

**Table 2. Inter-assay precision**

Patients	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

**Recovery**

A known amount of SHBG was added to three patient sera and the quantities recovered were measured. The results are shown in Table 3.

**Table 3. Recovery**

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

**Linearity (Dilution test)**

Four patient samples were diluted with Assay Buffer to 1:2, 1:5 and 1:10. SHBG values were assayed, and the results were corrected using dilution factors. Recovery results of these dilution tests are shown in Table 4.

**Table 4. Dilution of samples**

sample	Undiluted SHBG, nmol/l	Recovery %		
		1:2	1:5	1:10
1	58	100	107	97
2	85	100	117	108
3	120	102	100	102
4	185	89	95	96

### Specificity

Specificity of the SHBG ELISA TEST 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.

### High-dose hook effect

The assay was tested for a hook effect up to a SHBG concentration of 10000 nmol/l. No hook effect was observed.

### LITERATURE

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. (1990). Sex hormone binding globulin: origin, function and clinical significance. Ann. Clin. Biochem. 27: 532 - 541.

<b>Date Adopted</b>	<b>Reference No.</b>
<b>2005-09-27</b>	<b>DA-SHBG-2008</b>



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