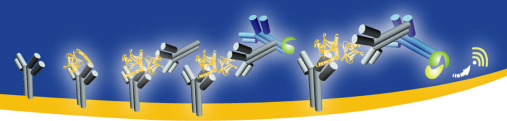
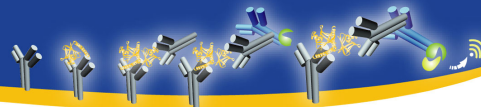


# **SHBG-ELISA**

***40-056-205043***



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# SHBG ELISA

40-056-205043

**RESEARCH USE ONLY**

## 1 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.

## 2 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.

## 3 MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipette with disposable plastic tips; 25µl (calibrators, 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

## 4 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.

## 5 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.

### 5.1 Dilution of Samples


Serum samples with SHBG concentrations greater than the highest calibrator 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")

## 6 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.

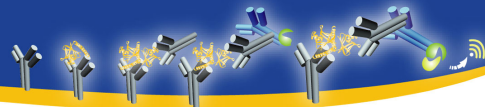
1.  **Breakpart Wells.** 96 wells on a plate coated with mouse monoclonal SHBG antibody, packed in a laminate bag. Ready for use.
2. 

ASS	BUF
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**Assay Buffer**, 1 x 80 ml. Ready for use.  
contains 0.05% Thimerosal, 0.02% Gentamicin as preservative.
3. 

CAL	N
-----	---

**SHBG Calibrators.** N= 0 to 4  
5 vials, 0.5 ml. Calibrated against human SHBG, WHO Standard (NIBSC 95/560). Exact calibrator values are given on the label of each vial.  
(See chapter 7.1 "Preparation of Reagents and Samples")  
contains 0.01% Thimerosal, 0.09% Gentamicin as preservative.



- |    |         |      |   |   |
|----|---------|------|---|---|
| 4. | CONTROL | N    | <p><b>Control</b>, N=1, 1 vial, 0.5 ml. (See chapter 7.1 "Preparation of Reagents and Samples") contains 0.01% Thimerosal, 0.09% Gentamicin as preservative.</p>                                  |   |
| 5. | Ab      | HRP  | <p><b>Enzyme Conjugate</b>, 14 ml. Ready for use.<br/>Mouse monoclonal SHBG antibody conjugated with horseradish peroxidase.<br/>contains 0.02% Thimerosal, 0.09% Gentamicin as preservative.</p> |   |
| 6. | WASH    | SOLN | CONC  | <p><b>Wash Solution</b>, 25 ml, (40x conc.). (See chapter 7.1 "Preparation of Reagents and Samples") contains 0.09% Thimerosal as preservative.</p> |
| 7. | CHROM   | TMB  |   | <p><b>TMB Substrate Solution</b>, 14 ml. TMB. Ready for use.<br/>H<sub>2</sub>O<sub>2</sub>/TMB</p>   |
| 8. | STOP    | SOLN |   | <p><b>Stop Solution</b>, 14 ml. Ready for use.<br/>0.25 M H<sub>2</sub>SO<sub>4</sub>. Avoid contact with eyes and skin.</p>                        |

## 7 TEST PROCEDURE

### 7.1 Preparation of Reagents and Samples

#### 1. Calibrators, controls, samples

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

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

#### 2. Wash Solution

Dilute the 40x concentrate in 975 ml distilled water.

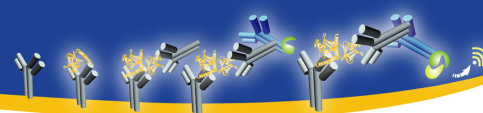
### 7.2 Assay Procedure

Allow all reagents to reach room temperature before use.

1. Mark the wells to be used on the plate.
2. Pipet 100 µl of Assay Buffer into each well.
3. Pipet 25 µl of diluted calibrators, 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 µl of washing solution.
6. Pipet 100 µl 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 300µl).
9. At timed intervals add 100 µl 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 µl 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.

## 8 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 calibration 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.



## 9 TEST PROCEDURE – SUMMARY

	Calibrators 0 – 4 (1:20)	Control Serum (1:20)	Samples (1:20)
Mark the strips			
Pipet Assay Buffer (µl)	100	100	100
Pipet diluted calibrators, 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 12 minutes at room temperature (20-25°C) for 8 minutes at room temperature (26°C and more)			
Stop solution (µl)	100	100	100
Mix			
Measure absorbance at 450 nm			

## 10 RESULTS

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

Example of worksheet and calibration 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	CAL 0 nmol/l	0.041		
3-4	CAL 4	0.096	0.055	
5-6	CAL 20	0.236	0.195	
7-8	CAL 75	0.621	0.580	
9-10	CAL 300	1.731	1.690	
11-12	Sample 1	0.203	0.162	16
13-14	Sample 2 (control)	0.461	0.420	54
15-16	Sample 3	1.153	1.102	190

### SHBG

