SHBG ELISA PROCEDURE SUMMARY

Total Assay Time - 60 min. (3 incubations: 30+15+15 min.)

	Allow **all** reagents to reach room temperature; arrange and label required # of strips. Prepare 1X solutions of enzyme conjugate (1:50 with assay buffer) and wash buffer (1:10 with water) as described in the instruction manual. **Dilute samples 1:10 with Standard A before use. Do not dilute Stds/calibrators.**
Step 1	Pipet **20 ul** of each **standard**, **control serum, and diluted serum samples** into appropriate wells *in duplicate*.
	Pipet **200 ul** assay buffer into **each** well.
	Cover the plate and incubate on a plate shaker (approx. 200 rpm) at room temperature for **30 minutes**
	Aspirate and **wash 3 X** with diluted (1:10 with water) wash buffer (300 ul/well/wash)
Step 2	Pipet **150 ul diluted Ab-enzyme conjugate** (**1:50** with assay buffer) into **each** well
	Cover the plate and incubate on a plate shaker (approx. 200 rpm) at room temp for **15 minutes.**
	Wash wells 3 X with diluted wash buffer (300 µl/well/wash)
Step 3	Pipet **150 ul HRP-substrate** solution into **each** well
	Cover the plate and incubate on a plate shaker at room temp for **15 minutes.**
	Pipet **50 ul stop** solution into **each well.** Measure absorbance at **450 nm**.

CHECK LIST (Check each box after completing each step)

	Step 1	Step 2	Step 3
Time: Start End			

KIT PROFILE

Date received: Cat # 700 Lot # ______ Exp. __________

Date kit opened _ Technician: ______________________________

Date used: ________ # Strips used __ # Remaining __________

Remarks ____

Instruction Manual No. M-700

Human Sex Hormone Binding Globulin (SHBG)

For Quantitative Determination of SHBG In Human Serum

Human SHBG ELISA KIT Cat. No. 700

Kit Contents: (reagents for 96 tests)

Components	Cat.#
Mouse Anti-h SHBG coated microwell strip plate (96 wells). Ready-to-use	701
SHBG Standard A, 0 nmol/L, 16 ml	702
SHBG Standard B, 3.3 nmol/L, 0.4 ml	703
SHBG Standard C, 12.5 nmol/L, 0.4 ml	704
SHBG Standard D, 55 nmol/L, 0.4 ml	705
SHBG Standard E, 160 nmol/L, 0.4 ml	706
SHBG Standard F, 295 nmol/L, 0.4 ml	707
SHBG Control, 0.4 ml (value printed on the vial)	700C
Lot Specific values of standards are printed on each vial. They should be used for plotting std. Curve. Calibrated against WHO 1st IS 95/560	
Assay Buffer, 40 ml	708
Anti-hSHBG-HRP Conjugate (**50 X** soln), 0.4 ml	709
Wash buffer concentrate (**10X** solution) 50 ml (dilute to 500 ml in distilled water)	W-10
HRP substrate soln, 16 ml	S-20
Stop solution, 6 ml	T-30
Complete Instruction Manual	M-700

Introduction

SHBG is a glycoprotein composed of 373 AA and 3 carbohydrate chains. It has affinity for steroids, especially the C18 or C19 and 17-beta-hydroxyl groups. The binding of steroids to SHBG is temperature and pH dependent. It is destroyed at 60oC or at pH lower than 5. The 3 steroids which have a high avidity for SHBG are: DHT, testosterone, and Estradiol. Many agents affect the SHBG levels: Estrogen and thyroid hormone increase the plasma concentration, androgen, GH, prolactin, insulin, obesity, and lipid decrease the concentration of plasma SHBG. Very small amounts of steroid are free in biological fluid (<2%); the remainder is mostly bound to SHBG and albumin. The free and bound steroids seem to exist in a state of dynamic equilibrium. The steroids bound to albumin have more than 95% of steroid binding sites unoccupied. It the case of SHBG in men about 40% are unoccupied while in female >75%. Changes in SHBG levels affects both albumin-bound and free steroid concentrations.

During pregnancy, there is a high level of SHBG and total testosterone but the free testosterone is decreased. Inversely, a decrease in the level of SHBG gives rise to an increase in albumin bound and free testosterone. In young male, SHBG is about 30 nmol DHT bound/L, while in female 50 nmol/L. SHBG increases until the eighties in both

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum concentration of SHBG detected using this assay is 0.2 nmol/l. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Four serum samples (SHBG concentrations 4.4, 16.2, 56.5, and 157 nmol/l) were run in sixteen replicates. The samples showed good intra-assay precision with %CV of 8.5, 4.3, 3.0 and 5.2, respectively.

Inter-assay precision:

Four serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (7.3-11.5 % CV). The actual values were: mean 3.8 nmol/l, SD 0.43 nmol/l, %CV 11.5; mean 19 nmol/l, SD 1.6 nmol/l, %CV 8.4; mean 63 nmol/l, SD 5.6 nmol/l, %CV 8.6; mean 194 nmol/l, SD 14, %CV 7.1, respectively.

3. RECOVERY

A known amount of SHBG (6.5, 28, and 165 nmol/l) was added to three patient sera (with original SHBG concentrations of 39, 60, and 157 nmol/l) and the total SHBG measured. The assay showed excellent mean recoveries of about 100% (range 92-113%).

4. LINEARITY -Four different patient samples (with original SHBG concentration of 58, 84, 120, and 184 nmol/l) were diluted (1:2, 1:5, and 1:10) with the assay buffer and their final SHBG values determined. The samples showed excellent mean recoveries of about 102% (range 96-108%).

5. SPECIFICITY - The specificity of SHBG ELISA kit was determined by measuring interference from high concentrations of TBG (thyroxine binding globulin). TBG concentrations of up to 500 mg/l had no effect on the SHBG assay.

6. HIGH DOSE HOOK EFFECT - SHBG concentrations of up to 1000 nmol/l did not cause any hook effect in this assay.

7. Species Crossreactivity- We have not checked the crossreactivity of this kit in other species (mouse, rat etc).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

Diluted enzyme conjugate should NOT be stored and used immediately. Reconstituted control serum is stable for one week at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Dilute (1:10) samples with standard A (e.g. 10 ul in a total volume of 90 ul std. A). **Dilute** wash buffer concentrate (1:10 with water) and Ab-enzyme conjugate (1:50 with assay buffer) as described above.

1. Label or mark the microtiter well strips to be used on the plate. Pipet **20 ul** of each standards, control, and diluted serum samples into appropriate wells in *duplicate*.
2. Pipet **200 ul of assay buffer** into **each** well. Mix gently. Cover the plate and incubate at room temperature for **30 minutes** on a **plate shaker** (about 200 rpm; failure to shake will decrease kinetics).
3. Aspirate and **wash the wells 3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **150 ul of *diluted* enzyme** conjugate into **each well**. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature on a **plate shaker** (about 200 rpm; failure to shake will decrease kinetics).
5. Aspirate and **wash the wells 3 X** with diluted wash buffer, as above.
6. Add **150 ul of HRP-substrate** at timed intervals into **each well.** Mix gently. Cover the plate and incubate at room temperature for **15 minutes** on a **plate shaker** (about 200 rpm; failure to shake will decrease color). Blue color develops.
7. Stop the reaction by adding **50 ul** of stopping solution to all wells at the same timed intervals as before. Mix gently. Blue color turns yellow.
8. Measure **Absorbance at 450 nm** within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 300 nmol/l SHBG must be diluted further (in addition to 1:10, as outlined in the procedure section) with the assay buffer and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each duplicate.
2. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples.
3. Draw the standard curve on logit-log graph paper by plotting net absorbance values of standards against appropriate SHBG concentrations. We recommend a 4-parameter logistic function. **Do not multiply the values by 10 if the samples were only diluted 1:10.** if samples were diluted more than 1:10 (e.g. 1:50) then the values be multiplied 1:5 and not 1:50.
4. Read off the SHBG concentrations of the control and patient samples.

Expected Normal Values

Group	N	Mean (nmol/L)	Range (nmol/L)
Adult Males	104	31	7-70
Adult Females	44	50	15-120

References: Rosner et al (1975) Biochemistry 14, 4813; Iqbal MJ et al (1979) J. Steroid Biochem. 10, 535; Adlind V et al (1982) Acta Endocrinol. 101, 248; Belgorosky A et al (1997) J Clin. Endo. Metabol. 64, 482; Longcope C et al (1987) J Clin. Endo. Metabol. 64, 513; Rittmaster RS et al (1987) An.. Int. Med. 23, 135; Reed MJ et al (1987) J Clin. Endo. Metabol. 45, 1211

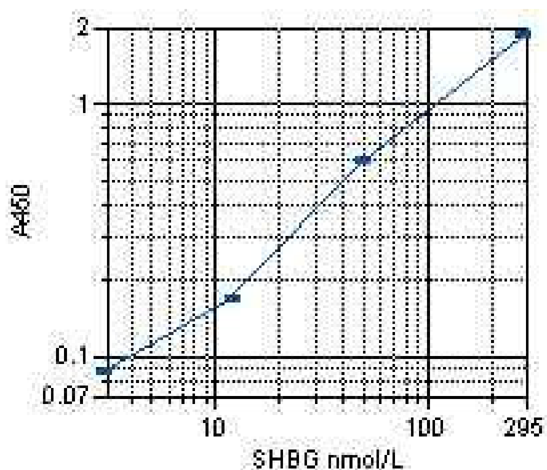
(2) Citations of ADI's SHBG (see web site for updated list)

Hryb Dj et al 2002 J. Biol. Chem, 277, 26618-26622;
Human LNCaP, DU 145, and PC 3 cells

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450} nm	Net Abs.	Calculated Conc. (nmol/l)
A1, A2	**Std. A** (0 nmol/l)	0.087		
B1, B2	**Std. B** (3.3 nmol/l)	0.187		
C1, C2	**Std. C** (12.5 nmol/l)	0.310		
D1, D2	**Std. D** (55 nmol/l)	1.083		
E1, E2	**Std. E** (160 nmol/l)	2.257		
F1, F2	**Std. F** (295 nmol/l)	2.755		
G1, G2	**Sample 1**	0.459	0.410	34
H1, H2	**Sample 2**	1.151	1.102	130

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

sexes. During the menstrual cycle, SHBG does not seem to vary appreciably. During pregnancy, SHBG rises rapidly until about the 30th week of gestation. SHBG is elevated by many drugs and various pathological conditions (hypogonadal men, hyperthyroidism, alcoholic liver disease, primary biliary cirrhosis, and anorexia in women). SHBG is decreased in myxedema, hyperprolactinemia, acromegaly, GH therapy, obesity, and congenital adrenal hyperplasia.

ADI's SHBG ELISA kit is a sandwich type ELISA kits designed to measure SHBG in human serum. The assay is highly sensitive (needs 2 ul sample) and requires just 1 hour.

PRINCIPLE OF THE TEST

SHBG ELISA kit is based on sequential binding of human SHBG from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of SHBG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International SHBG ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20ºC for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.