Quantikine® ELISA

Human SHBG Immunoassay

Catalog Number DSHBG0B

For the quantitative determination of human Sex Hormone Binding Globulin (SHBG) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human SHBG (sex hormone binding globulin), also known as ABP (androgen-binding protein) is a secreted, non-disulfide linked homodimer that belongs to the SHBG family (1-5). Members of this small family have tandem repeats of 170 amino acid (aa) laminin G-like domains. Each SHBG monomer is a 47-53 kDa, 373 aa glycoprotein that contains a steroid-binding site, a dimerization site, and a membrane binding site in its N-terminal laminin G-like domain (4-8). Human SHBG shares approximately 67% aa sequence identity with mouse and rat SHBG. Male and female sex hormones are bound with similar high affinity (8). SHBG is mainly synthesized in the liver by hepatocytes and circulates in blood, while ABP (SHBG that is found in the testes) is synthesized by Sertoli cells and circulates in the male reproductive system (2). SHBG can be found intracellularly, and synthesis of intracellular SHBG has been demonstrated in normal and transformed cells of the prostate, placenta, endometrium, and some breast tissues (4, 9-11). Usage of one O-linked glycosylation site near the N-terminus and two N-linked sites near the C-terminus per monomer is variable, differs between SHBG and ABP, and can affect half-life, secretion, and membrane binding (4, 10-13). At least some glycosylation is necessary for efficient secretion of SHBG, but not for hormone binding (4, 5, 10).

SHBG is the main regulator for bioavailability of sex hormones, but this regulation potentially involves several different mechanisms depending on the hormones, cells and tissues involved (4). One line of evidence shows that high-affinity hormone binding by SHBG blocks uptake of the hormones by target cells. In another line of evidence, SHBG binding to megalin, an endocytic receptor in reproductive tissues, facilitates cellular uptake of bound androgens and estrogens from the circulation (4, 14). Megalin-independent binding of SHBG to cells also occurs, but only when hormones are not bound (9, 11). Once bound to cells, however, SHBG may bind estradiol (for example), initiating intracellular signaling through cAMP and slowing cell growth (4, 13, 15). Opposing the cAMP signaling, free testosterone and some estrogen and testosterone metabolites promote anti-apoptotic signaling through the MAPK/ERK pathway (4, 11, 13). Extracellularly, fibulins can bind and sequester SHBG within the extracellular matrix, especially when it is carrying estradiol (4, 12, 16).

Post-menopausal women with estrogen receptor-positive breast cancers frequently show low circulating SHBG, while high SHBG correlates with reduced breast cancer risk, presumably due to lower bioavailability of estrogens (4). Low circulating SHBG (coupled with low plasma testosterone in males) is correlated with high body mass index, metabolic syndrome and type 2 diabetes (17-21). The influence of metabolism-related transcription factors, such as PPARγ, on transcription of SHBG in the liver links SHBG expression with metabolic syndrome (22). A common SHBG polymorphism (D356N) that creates an additional N-linked glycosylation site increases half-life, plasma concentration and cell binding of hormone-free SHBG, and reduces breast and endometrial cancer risk in postmenopausal women (4, 15, 20, 21, 23, 24). Another polymorphism conferring 8 repeats or more of a 5′ adenosine repeat sequence is associated with lower circulating SHBG in most studies and, in females, higher risk of polycystic ovary disease and coronary artery disease (4, 17, 21, 23, 25, 26).

The Quantikine Human SHBG Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human SHBG in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human SHBG and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human SHBG showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human SHBG.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human SHBG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SHBG present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human SHBG is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SHBG bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human SHBG Microplate	893886	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human SHBG.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human SHBG Standard	893888	2 vials of recombinant human SHBG in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new Standard for each assay. Discard after use.
Human SHBG Conjugate	893887	21 mL of monoclonal antibody specific for human SHBG conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-75	895811	11 mL of buffered animal serum with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-24	895325	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:15 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12.5 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12.5 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human SHBG Controls (optional; R&D Systems, Catalog # QC158B).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-24 (diluted 1:15).*

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

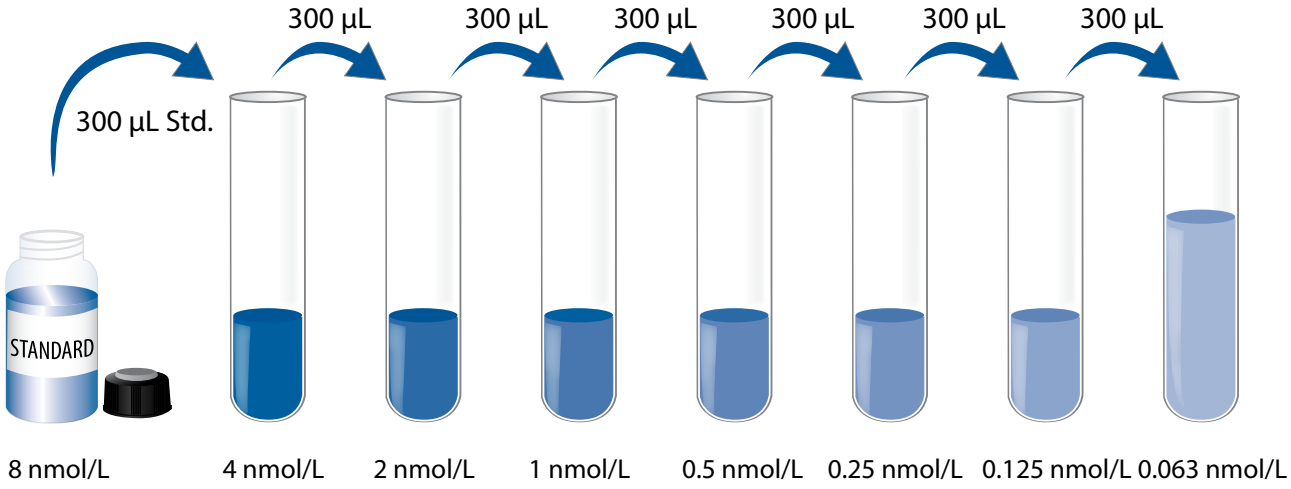
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-24 (diluted 1:15) - Add 4 mL of Calibrator Diluent RD5-24 to 56 mL of deionized or distilled water to prepare 60 mL of Calibrator Diluent RD5-24 (diluted 1:15).

Human SHBG Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human SHBG Standard with deionized or distilled water. This reconstitution produces a stock solution of 8 nmol/L. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD5-24 (diluted 1:15) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4 nmol/L standard serves as the high standard. Calibrator Diluent RD5-24 (diluted 1:15) serves as the zero standard (0 nmol/L).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-75 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human SHBG Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

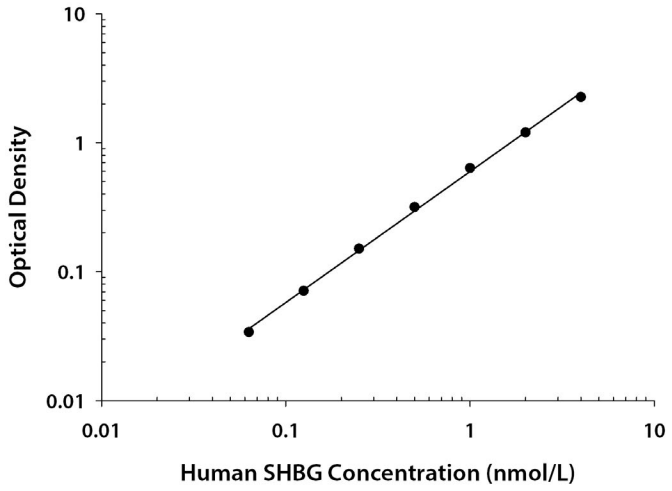
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human SHBG concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(nmol/L)	O.D.	Average	Corrected
0	0.010 0.010	0.010	—
0.063	0.043 0.045	0.044	0.034
0.125	0.079 0.082	0.081	0.071
0.25	0.160 0.162	0.161	0.151
0.5	0.325 0.329	0.327	0.317
1	0.641 0.653	0.647	0.637
2	1.190 1.233	1.212	1.202
4	2.263 2.277	2.270	2.260

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using one lot of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (nmol/L)	0.565	1.36	2.72	0.562	1.35	2.71
Standard deviation	0.032	0.040	0.097	0.042	0.079	0.131
CV (%)	5.7	3.0	3.6	7.6	5.8	4.8

RECOVERY

The recovery of human SHBG spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	92	87-96%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human SHBG were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	106	105	101	103
	Range (%)	104-108	100-112	96-104	99-111
1:4	Average % of Expected	110	105	96	100
	Range (%)	107-113	102-112	92-101	97-105
1:8	Average % of Expected	115	103	91	99
	Range (%)	113-117	101-106	88-93	95-106
1:16	Average % of Expected	111	95	83	87
	Range (%)	108-114	90-97	75-88	81-91

* Samples were diluted prior to assay.

SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of human SHBG ranged from 0.001-0.012 nmol/L. The mean MDD was 0.006 nmol/L.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human SHBG produced at R&D Systems.

The recombinant protein is directly calibrated to the NIBSC/WHO International Standard 08/266.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human SHBG in this assay. No medical histories were available for the donors used in this study.

Male Samples	Mean (nmol/L)	Range (nmol/L)	Standard Deviation (nmol/L)
Serum (n=16)	30.1	13.2-51.2	11.8
EDTA plasma (n=16)	32.6	13.6-50.9	12.6
Heparin plasma (n=16)	31.0	11.9-49.3	11.9

Female Samples	Mean (nmol/L)	Range (nmol/L)	Standard Deviation (nmol/L)
Serum (n=19)	101	12.9-273	66.7
EDTA plasma (n=19)	110	16.9-321	79.6
Heparin plasma (n=19)	104	15.7-282	68.9

Cell Culture Supernates - Aliquots of cell culture supernates from 16 various cell lines were assayed for levels of human SHBG. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human SHBG.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human SHBG control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Caspr2
Contactin
Gas6
Serpin A7

Recombinant mouse:

Gas6

Other Factors:

Androsterone
Androstenedione
Epitestosterone
Estradiol
Progesterone
Testosterone
trans-Dehydroandrosterone

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