Human SHBG ELISA Kit

Enzyme Immunoassay for the quantitative determination of human SHBG in serum and plasma

Catalog number: ARG80853

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Sex-hormone-binding globulin (SHBG) is a B-globulin that specifically binds steroid hormones. 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.

In general the measurement of SHBG offers the possibility for calculating the free fraction of vatious steroid hormones based on the measurement of the total concentration in serum. But there is also a method available for the direct measurement of free steroid hormones in saliva. The metabolic activity of any steroid in any case is just represented by the free hormone fraction which represents less than 5% of the total. The non-invasive sampling of saliva samples offers another advantage of salivary testing.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for SHBG has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any SHBG present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for SHBG is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) 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 by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of SHBG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP- SHBG antibody Conjugate	14 ml (ready to use)	4°C
Standards 0-6 (0, 4, 16, 32, 65, 130, 260 nmol/L)	7 X 0.5 ml	4°C
Control Low (19.6 nmol/L; acc. range: 12.9 – 26.9 nmol/L)	0.5 ml	4°C
Control High (75.3 nmol/L; acc. range: 45.7 – 95.0 nmol/L)	0.5 ml	4°C
Assay Buffer	125 ml (ready to use)	4°C
40X Wash Buffer	30 ml	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- When stored at 2- 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2- 8 °C. Microtiter wells must be stored at 2- 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 2 months if stored as described above.
- 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.
- Briefly spin down the HRP-SHBG antibody conjugate before use.
- If crystals are observed in the 40X Wash buffer and Assay buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- 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.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after

completing the rinsing steps.

- 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.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Once the test has been started, all steps should be completed without interruption.
- 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.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Collect serum and assay immediately or aliquot and store samples at ≤ -20°C for up to 3 months. Samples should be frozen only once at-20 °C prior to assay. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate 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 for up to 3 months. Samples should be frozen only once at-20 °C prior to assay. Avoid repeated freeze-thaw cycles.

Note:

1. EDTA and citrate plasma samples may give slightly lower results.
2. Prior to assay, thawed samples should be inverted several times prior to testing
3. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. 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 1 weeks at room temperature.
- Standard, controls: Prior to the assay, all standards and controls need to be diluted 1+100 in Assay Buffer. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

  Example: 10 µL standard (control) + 1000 µL Assay Buffer
- Sample: Prior to the assay, all samples need to be diluted 1+100 in Assay Buffer (10 µL sample + 1000 µL Assay Buffer). Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

  If the initial assay found samples contain SHBG higher than the highest standard, the samples can be diluted with Assay Buffer and then re-assay the samples.

  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example for further dilution:

  a) Dilution 1:10: 10 µL Serum + 90 µL Assay Buffer (mix thoroughly).

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

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **prediluted Standard, controls and samples** in duplicate into the appropriate wells. Incubate for **120 minutes at RT.**
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with distilled water (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels. (The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!)
4. Add **100 µl** of **HRP- SHBG antibody** into each well. Incubate for **30 minutes at RT**.
5. Aspirate and **wash each well as step 3**.
6. Add **100 µl** of **TMB substrate** to each well. Incubate for **15 minutes at room temperature** in dark.
7. Add **100 µl** of **Stop Solution** to each well.
8. Read the OD with a microplate reader at **450 nm** immediately. It is recommended read the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear, semi-log or log-log 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.

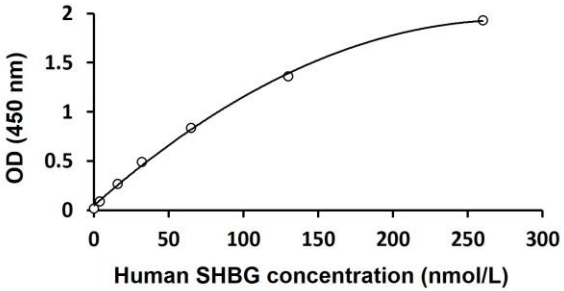
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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 generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 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.

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

Specificity

The following substances were tested for cross reactivity of the assay:

Corticoid binding globulin < 0.2%

Thyroxin binding globulin < 0.04%

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 3.73% and the CV value of inter-assay precision was 5.85%.

Interferences

Haemoglobin, bilirubin and triglyceride have no influence on the assay results.

Recovery

87.4-99.8%

Linearity

100.1-101.6%