EAGLE BIOSCIENCES

Sex Hormone Binding Globulin (SHBG) ELISA Assay Kit

Catalog Number:
SHB31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.0 (30 OCT 23)

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INTENDED USE

The Eagle Biosciences Sex Hormone Binding Globulin (SHBG) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Sex Hormone Binding Globulin by an enzyme immunoassay in human serum. The Eagle Biosciences Sex Hormone Binding Globulin (SHBG) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Sex hormone binding globulin (SHBG) is a glycoprotein composed of 373 amino acid residues and three carbohydrate side chains. SHBG has been known by many other names including Testosterone estradiol Binding Globulin (TeBG), Sex steroid Binding Protein (sBP) and Sex Steroid Binding Globulin (SSBG). One of the main properties of SHBG is its high affinity for steroids, especially the C18, C19 and 17α-hydroxyl groups. The binding of steroids to SHBG is temperature and pH dependent. The three steroids that have a high avidity for SHBG are Dihydrotestosterone, Testosterone and Estradiol. Very small amounts of these steroids are free in biological fluid; the majority are bound to SHBG and albumin. These two fractions, that is, free and bound exist in a state of dynamic equilibrium. When the level of SHBG concentration changes, a remarkable change occurs in both albumin-bound hormone and also in the free fraction.

Throughout life SHBG increases until the eighties in both sexes. During the menstrual cycle SHBG does not seem to vary appreciably, however, according to some authors the concentration of SHBG is elevated in the luteal phase. During pregnancy the level of SHBG rises rapidly until about the 30th week of gestation.

PRINCIPLE OF THE ASSAY

The principle of the Eagle Biosciences Sex Hormone Binding Globulin (SHBG) ELISA Assay Kit follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for SHBG is immobilized onto the microplate and another monoclonal antibody specific for a different region of SHBG is conjugated to horse radish peroxidase (HRP). SHBG from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of SHBG in the sample. A set of standards is used to plot a standard curve from which the amount of SHBG in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >295 nmol/L. If further dilution and retesting is required, only the SHBG assay buffer may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.

26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of SHBG in human serum. The kit is not calibrated for the determination of SHBG in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is intended for research use only and should not be used for diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL
The reagents should be considered a potential biohazard and handles with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain materials of human origin. Donors have been tested and found to be negative for the presence of HBsAg, antibodies to HIV ½ and antibodies to HCV. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Dilute patient serum samples 1:10 in assay buffer before use.

1. Pipette 90µL of Assay Buffer into a new polypropylene microcentrifuge tube or HDPE tube.
2. Pipette 10 µL of the serum specimen into the tube from step 1 that contains 90µL of assay buffer
3. Close the tube and label with specimen identification information.
4. Mix the conents of the tube by vortexing

***Do not dilute the standards and controls, they are ready for use.**

Note: Different volumes of the Assay Buffer and serum may be used provided that the required 1:10 ratio is maintained (1 part serum specimen to 9 parts Assay buffer)

Pre-treated serum specimens must be assayed on the same day as they were prepared. Do not store pre-treated serum specimens beyond this time limit.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 10, 20, 50, 90, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 13)
6. Polypropylene or HDPE tubes for sample pre-treatment
7. Vortex Mixer

REAGENTS PROVIDED

1. **Mouse Anti-SHBG Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   - Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **Mouse Anti-SHBG Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate** — Requires Preparation X51
   - Contents: Anti-SHBG monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 0.4 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:51 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 300 µL of HRP in 15 mL of assay buffer. Discard any that is left over.

3. **SHBG Calibrators** — Ready to Use
   - Contents: Six vials containing SHBG in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of SHBG.

   Calibrated against World Health Organization (WHO) 1st IS 95/560.

   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 nmol/L	0.4 mL/vial
Calibrator B	3.3 nmol/L	0.4 mL/vial
Calibrator C	12.5 nmol/L	0.4 mL/vial
Calibrator D	55 nmol/L	0.4 mL/vial
Calibrator E	160 nmol/L	0.4 mL/vial
Calibrator F	295 nmol/L	0.4 mL/vial

Storage: Refrigerate at 2–8°C.
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** — Ready to Use
   Contents: Two vials containing S HBG in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of SHBG. Refer to vial labels for the acceptable range.
   Volume: 0.4 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.
5. **Wash Buffer Concentrate** — Requires Preparation X10
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water
6. **Assay Buffer** — Ready to Use
   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Volume: 55 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
7. **TMB Substrate** — Ready To Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
8. **Stopping Solution** — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: Dilute 1:10 With Assay Buffer Before Use.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-SHBG-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control and diluted specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 200 µL of assay buffer into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
8. Incubate on a plate shaker (approximately 200 rpm) for 15 minutes at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 150 µl of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
12. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.
    * If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. **If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended**.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 295 nmol/L, then dilute it with assay buffer at a dilution of no more than 1:10 (from original 1:10 dilution). The result obtained should be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate.
2. The calibrator with the highest concentration meets the optical density acceptable range as stated in the QC certificate.

3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external control that were used meet the acceptable ranges.

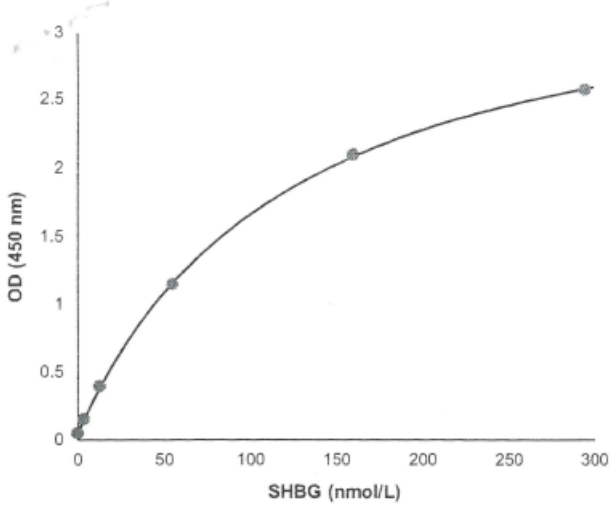
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (nmol/L)
A	0.051	2	0
B	0.151	6	3.3
C	0.394	15	12.5
D	1.152	45	55
E	2.099	81	160
F	2.579	100	295
Unknown	0.576	-	21

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.