Sex Hormone Binding Globulin (SHBG) 
Test System
Product Code: 9125-300

1.0 INTRODUCTION

1.1 Overview: The Quantitative Determination of Sex Hormone Binding Globulin (SHBG) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been tested for the presence of HIV, Hepatitis B and C antibodies, and no evidence of infection by these agents has been found. Human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory practice and standard procedures can be found in the Center for Disease Control / National Institute of Health, “ Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition (1992) (CDC): 88-8935.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

4.0 REAGENTS

Materials Provided:

A. SHBG Calibrators – 1ml/vial - Icons A-F

Six (6) vials of human serum reference calibrators at concentrations of 0 (A), 10 (B), 25 (C), 50 (D), 100 (E) and 250 (F) nmol/L. A preservative has been added. Store at 2-8°C.

B. Enzyme Reagent – 12ml/vial - Icon \( \text{C} \)

One (1) vial of anti-human SHBG-DHP and biotinylated anti-human SHBG presented in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. SHBG Diluent – 60ml/vial – Icon \( \text{U} \)

One (1) vial of a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate – 96 wells – Icon \( \text{O} \)

One 96-well plate pre-coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon \( \text{H} \)

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon \( \text{S} \)

One (1) vial containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon \( \text{S} \)

One (1) vial containing hydrogen peroxide (H₂O₂) in acetate buffer. Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for 15 days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Microplate reader with a 450nm absorbance capability.
3. Microplate reader with a 450nm absorbance capability.
4. Microplate reader with a 450nm absorbance capability.

5. Absorbent Paper for blotting the microplate wells.

6. Plastic wrap or microplate cover for cleaning steps.

7. Vacuum aspirator (optional) for wash steps.

8. Timer.


5.0 PRECAUTIONS

**Test procedure should be performed by a skilled individual or trained professional**

1. Format the microwells’ wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette the appropriate serum reference calibrator, control or patient sample* into the assigned well.

3. Add 0.050ml (50µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time.

4. Swirl the microplate gently for 20-30 seconds.

5. Incubate 30 minutes at room temperature.

6. Plot the absorbance of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or mechanical plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Cal 1 (0.100ml/100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time.

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 690-630nm to account for interferences) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

6.0 SPECIMEN COLLECTION AND PREPARATION

1. Blood samples should be collected in a clean, dry vacutainer® tube containing an appropriate anticoagulant. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

2. Plot the absorbance for each duplicate serum reference versus the horizontal axis of the graph (the duplicates of the unknown point on the curve, and read the concentration (in nmol/L) from the corresponding SHBG concentration in nmol/L on linear scale.

3. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

1. Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls include positive and negative controls, and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied controls. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash to 1000ml with distilled or deionized water in a suitable storage container. Store diluted wash at 2-30°C for up to 60 days.

2. Working Substrate Solution – Stable for one (1) year

Pour the contents of the amber vial labeled Solution A’ into the clear vial labeled Solution B’. Place yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

3. Patient Sample Dilution (1/21)

Dispense 0.025ml (25µl) of each patient specimen into 0.050ml (50µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have become cloudy.

9. TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators, controls and patient specimens to be assayed to room temperature (20 - 27°C).

1. Pipette the appropriate serum reference calibrator, control or patient sample* into the assigned well.

2. Pipette 0.050ml (50µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time.

3. Swirl the microplate gently for 20-30 seconds.

4. Incubate 30 minutes at room temperature.

5. Plot the absorbance of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

6. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or mechanical plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

7. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time.

8. Incubate at room temperature for fifteen (15) minutes.

9. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.

10. Read the absorbance in each well at 450nm (using a reference wavelength of 690-630nm to account for interferences) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

11. Plot the absorbance for each duplicate serum reference versus the horizontal axis of the graph (the duplicates of the unknown point on the curve, and read the concentration (in nmol/L) from the corresponding SHBG concentration in nmol/L on linear scale.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

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<th>Sample ID</th>
<th>Well</th>
<th>Abs</th>
<th>Mean</th>
<th>Abs (B)</th>
<th>Conc (nmol/L)</th>
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<td>2.658</td>
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</tbody>
</table>

1. The calculation of the average absorbance of the duplicates for each unknown should be made as described in the “procedure for handling blood products can be found in the Center for Disease Control / National Institute of Health, “ Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition (1992) (CDC): 88-8935.

2. The results should be read within thirty (30) minutes of adding the stop solution.
11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator ‘A’ should be < 0.05
2. The absorbance (OD) of calibrator ‘F’ should be 2.1.3
3. Four out of six quality control pools should be within the established ranges.
4. The absorbance (OD) of calibrator ‘A’ should be < 0.05
11.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.0 ASSAY PERFORMANCE

1.9. Assay Performance
1.10. Expected Ranges of Values

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

In order to validate the intra-assay precision of the SHBG AccuBind® ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 1.5 to 2.6% was obtained.

14.2 Sensitivity

The SHBG AccuBind® ELISA test system has a sensitivity of 0.0152nmol/L. The sensitivity was ascertained by determining the variability of the 0 nmol/L serum calibrator, and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The SHBG AccuBind® ELISA test system was compared with a reference method. Biological specimens with SHBG levels in the low, medium and high ranges were used; the values ranged from 4.6 to 184.0 nmol/L. The total number of such specimens was 60. The least square regression equation and the correlation coefficient were computed for this SHBG AccuBind® ELISA method in comparison with the reference method (Table 3).