Monobind Inc.
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AccuLite
CLIA Microwells

# Sex Hormone Binding Globulin (SHBG) Test System

***Product Code: 9175-300***

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Sex Hormone Binding Globulin (SHBG) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence.**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

In order for the sex-steroids testosterone, 5α-dihydrotestosterone, and 17β-estratdiol to reach the tissues, a transport molecule known as the Sex Hormone Binding Globulin (SHBG) is used. No more than 10% of these steroids are actually in plasma unbound and therefore biologically active. This protein's steroid binding capacity is highly dependent on the temperature and pH of its environment.[1,2,3] Originating in the liver, this glycoprotiein is a 93.4 kDa homodimer. Overall, SHBG is responsible for the balance of steroid levels and, in certain instances, has an influence on the effects these steroids have on their targets. The serum levels of this globulin have been shown to vary drastically between individuals, even if considered to be in a "normal" state of health.[2,3,4]

Physiological status changes due to hormonal, metabolic, and nutritional factors are reflected in the concentration of SHBG in serum. Rises in oestrogens correlate with an increase in SHBG, whereas a rise in androgens inhibits SHBG production. Aging and conditions like polyscystic ovarian syndrome demonstrate this effect.[2,3,5] SHBG levels also correlates strongly with conditions like hyperthyroidism, insulin resistance, central adiposity, and dyslipedemia. More importantly, low levels of SHBG show an increased risk for diabetes and cardiovascular disease.[5,6,7]

SHBG is also used to calculate the Free Androgen Index (FAI). The calculation method has been applied frequently to the determination of Free Testosterone (FT) levels. In the past, analog methods and dialysis have been used to obtain free testosterone levels, but each has a very striking downfall; Analog methods tend to give values substantially lower than the actual clinical status and dialysis is a laborious technique. This calculated method uses SHBG, total testosterone(T) and albumin concentrations to calculate the free Testosterone in human serum.[8,9]

## 3.0 PRINCIPLE

**Immunoenzymometric assay (TYPE 3):**
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-SHBG antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(m)} + Ag_{SHBG} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{SHBG} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{SHBG}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(m)}$ = Enzyme -Monoclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(m)} - Ag_{SHBG} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(m)} - Ag_{SHBG} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the well surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Materials Provided:**

**A. SHBG Calibrators – 1.0 ml/vial - Icons A-F**
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 10 (**B**), 25 (**C**), 50 (**D**), 100 (**E**) and 250 (**F**) nmol/L. Store at 2-8°C. A preservative has been added. (The Calibrators are standardized against WHO's 2nd IS 08/266 for SHBG).

**B. SHBG Tracer Reagent – 12 ml/vial - Icon**
One (1) vial of anti-human SHBG-HRP incorporated complex in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

**C. SHBG Diluent – 60 ml/vial – Icon**
One (1) vial of a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

**D. Streptavidin Plate – 96 wells – Icon**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**E. Wash Solution Concentrate – 20ml. - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**F. Signal Reagent A – 7ml/vial - Icon $C^A$**
One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

**G. Signal Reagent B – 7ml/vial - Icon $C^B$**
One (1) vial containing hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C (see Reagent Preparation Section).

**H. 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 sixty (60) days when stored at 2-8ºC. 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. Dispenser(s) for repetitive deliveries of 0.100 & 0.300ml (100 & 300µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

## 5.0 PRECAUTIONS

***For In Vitro Diagnostic Use***
***Not for Internal or External Use in Humans or Animals***

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures 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, 1988, HHS Publication No. (CDC) 88-8395.

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

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

**In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.**

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 use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. 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 concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2-8 °C
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips. A slight excess of solution is made. **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.
3. **Patient Sample Dilution (1/21)**
   Dispense 0.050ml (50µl) of each patient specimen into 1ml 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 bacteria growth.***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).*
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference, control* or patient sample* into the assigned well.
   ***Patient sample dilution required – see section 8.0 Reagent Preparation.***
3. Add 0.100ml (100µl) of the SHBG Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl (0.350ml) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual 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 four (4) additional times.**
8. Add 0.100 ml (100µl) of working signal solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
   **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**
9. Incubate at room temperature for five (5) minutes.
10. Read the Relative Light Units (RLUs) in each well with a chemiluminescence Microplate reader for 0.5-1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal solution.**

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of SHBG in unknown specimens.
1. Record the RLUs (*Relative Light Units*) obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference calibrator versus the corresponding SHBG concentration in nmol/L on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of SHBG for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in nmol/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU's (23388) of the unknown intersects the calibration curve at (51.5 nmol/L) SHBG concentration (See Figure 1)*.

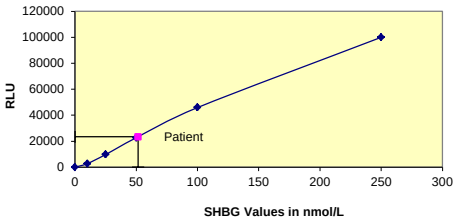
**Note:** Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**EXAMPLE 1**

| Sample I.D. | Well | Abs | Mean Abs (B) | Conc |
|---|---|---|---|---|
| Cal A | A1 | 10 | 11 | 0 |
| | B1 | 11 | | |
| Cal B | C1 | 2739 | 2776 | 10 |
| | D1 | 2812 | | |
| Cal C | E1 | 9846 | 9765 | 25 |
| | F1 | 9684 | | |
| Cal D | G1 | 21784 | 22719 | 50 |
| | H1 | 23654 | | |
| Cal E | A2 | 45656 | 45917 | 100 |
| | B2 | 46178 | | |
| Cal F | C2 | 99990 | 100000 | 250 |
| | D2 | 100009 | | |
| Patient 1 | A3 | 24187 | 23388 | 51.5 |
| | B3 | 22588 | | |

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

**Figure 1**




## 11.0 Q.C. PARAMETERS

**In order for the assay results to be considered valid the following criteria should be met:**

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

## 12.0 RISK ANALYSIS

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

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

### 12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Serum SHBG concentration is dependent upon a multiplicity of factors. Total SHBG concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.

## 13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the SHBG AccuLite® CLIA Test System are detailed in Table 1.

**TABLE 1**
**Expected Values for the SHBG AccuLite® CLIA test system**

| POPULATION | RANGE |
|---|---|
| Males | 10 – 57 nmol/L |
| Females (non-pregnant) | 18 – 144 nmol/L |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

In order to validate the intra-assay precisions of the SHBG AccuLite® CLIA 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 1.5to 2.6% was obtained.

**TABLE 2**
**Intra-Assay Precision (in nmol/L)**

| SAMPLE | N | X | σ | C.V.% |
|---|---|---|---|---|
| Control Level 1 | 24 | 19.366 | 1.012 | 5.2 |
| Control Level 2 | 24 | 51.395 | 3.441 | 6.7 |
| Control Level 3 | 24 | 89.754 | 7.182 | 8.0 |

### 14.2 Sensitivity

The SHBG AccuLite® CLIA test system has a sensitivity of 0.0132nmol/L. The sensitivity was ascertained by determining the variability of the 0 IU/ml serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

### 14.3 Accuracy

A method comparison between the Monobind AccuLite® and AccuBind® diagnostic kits was performed. Biological specimens from low, normal, and high SHBG level populations were used; the values ranged from 20.43 nmol/L to 369.16 nmol/L. Overall, a total of 30 specimens were evaluated. The least square regression equation and the correlation coefficient were computed for the AccuLite® method when compared to the reference method. Monobind requires a high level of correlation and a target value for the correlation coefficient greater than 0.9 when developing products.

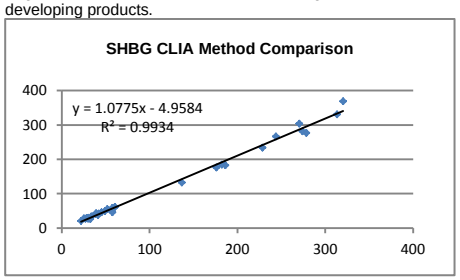



**TABLE 4**

| Parameter | Monobind | Reference |
|---|---|---|
| Low | 20.43 | 22.15 |
| High | 369.16 | 320.41 |
| Mean | 116.77 | 112.98 |
| Intercept | -4.9584 | |
| Slope | 1.0775 | |
| Corr ($R^2$) | 0.9934 | |

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### 14.4 Specificity

The specificity of the SHBG AccuLite® CLIA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix.

**SHBG ELISA Specificity and Cross Reactivity**

| SUBSTANCE | Cross Reactivity |
|---|---|
| Corticosteroid Binding Globulin | < 0.3 |
| Thyroxine Binding Globulin | < 0.08 |

## 15.0 REFERENCES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**Effective Date: 2019-Jul-16** **Rev 1** **DCO: 1353**
**MP9175** **Product Code: 9175-300**


| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 1ml set |
| | B) | 1 (12ml) | 2 (12ml) |
| | C) | 1 (60ml) | 2 (60ml) |
| | D) | 1 plate | 2 plates |
| | E) | 1 (20ml) | 1 (20ml) |
| | F) | 1 (7ml) | 2 (7ml) |
| | G) | 1 (7ml) | 2 (7ml) |


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**Please visit our website to learn more about our products and services.**

**Glossary of Symbols**
(EN 980/ISO 15223)

- IVD — In Vitro - Diagnostic Medical Device
- Temperature Limitation Storage Condition (2-8° C)
- Consult Instructions for Use
- REF — Catalogue Number
- Contains Sufficient Test for Σ
- LOT — Batch Code
- Used By (Expiration Day)
- Date of Manufacturer
- Manufacturer
- EC REP — Authorized Rep in European Country
- CE — European Conformity