

























SHBG ELISA

**Enzyme Immunoassay for Quantitative Determination of
Sex Hormone-Binding Globulin in Human Serum**

Instructions for use

1. SYMBOL LEGEND

	In vitro diagnostic medical device		Batch code
	Catalogue number		Manufacturer
	Use by		Consult operating instructions
	Date of manufacture		Biological risks
	Temperature limitation		Conjugate
	Coated microplate (96 wells)		Substrate
	Wash solution, 20X concentrated		Stop solution
			
	Calibrators		Sample diluent
	Control		Optical density
	Contains sufficient for <n> tests		Deionized or distilled water
	Reconstitute with specified volume of liquid		

2. INTENDED USE

SHBG kit is provided for the **quantitative determination of sex hormone-binding globulin (SHBG) in human serum.**

SHBG is a serum protein that binds steroid sex hormones. SHBG is synthesized in the liver. It is a dimeric glycoprotein with a molecular mass of 80 to 100 kDa (depending on the degree of glycosilation). Each dimer binds one molecule of steroid. The protein is specific for 5-alpha-dihydrotestosterone, testosterone, and 17-beta-estradiol.

Steroid hormones circulate in blood partly in their free form and partly as a complex with albumin or corresponding specific protein (SHBG in the case of sex steroids). Fraction of sex steroid hormone that is not bound to SHBG (so-called bioavailable hormone) can perform its regulatory function in target cells. Steroids bound to albumin are also bioavailable, because such binding is weak and does not block the diffusion of the hormone into target cells. SHBG is the primary carrier protein for sex steroid hormones. It participates in plasma metabolic clearance rate of steroid hormones. Thus, the level of SHBG regulates the bioavailability of these hormones to target tissues.

SHBG shows maximal affinity to androgens and less – to estrogens. SHBG synthesis is affected by sex steroids. Estrogens show stimulating effect, whereas androgens inhibit its synthesis.

In healthy individuals SHBG concentration may vary over a wide range. In general, its concentration in females is higher than in males. SHBG concentration increases in the case of hyperthyroidism or hyperestrogenia (luteal phase of the cycle, pregnancy, intake of estrogen-containing oral contraceptives, cyrrhosis etc.). In adult males the concentration of bioavailable testosterone decreases with age due to increased concentration of SHBG. In females decreased concentration of SHBG correlates with hyperandrogenic status (polycystic ovary syndrome, congenital dysfunctions of adrenal cortex, hirsutism). Moderate decrease in SHBG concentration is also observed in the case of hypothyroidism, Cushing disease, hyperprolactinemia, acromegalia and after the treatment with androgens or progestins that have androgenic effect.

SHBG concentration is used for calculation of free androgen index (ratio between concentrations of total testosterone and SHBG).

3. PRINCIPLE OF TEST

SHBG kit is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of SHBG molecule. One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. SHBG molecules from the serum sample bind to both immobilized antibody and anti-SHBG-peroxidase conjugate. Then the wells are washed with wash solution to remove any material not bound to the inner surface of the wells. Quantity of the bound conjugate is directly proportional to SHBG level in the sample.

During the incubation with TMB solution the colour is developing. The intensity of the colour is directly proportional to SHBG concentration in specimens. SHBG concentration in the patient sample is read from a standard curve that is processed in each assay.

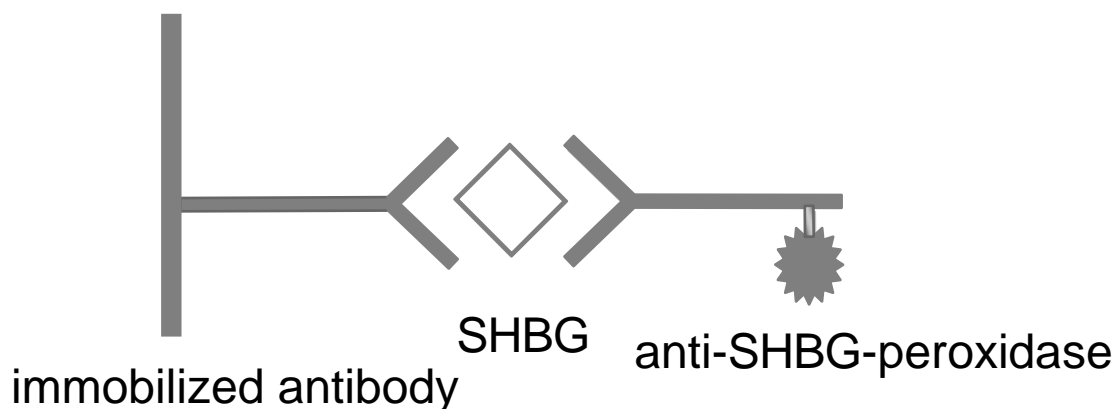


Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

The expiration date of the kit is printed on the box label; the expiration date for each component is printed on the respective label.

SHBG kit should be stored at +2...+8 °C upon receipt, preferably in the original kit box, until the expiration date. Storage at +25 °C is allowed but for no more than 5 days.

Shelf life of the kit is 18 months.

After initial opening the kit is stable till expiry if stored at +2...+8 °C.

If used in several separate experiments, after initial opening kit contents should be stored as follows:

- unused strips: in a firmly closed resealable zipper bag at +2...+8 °C until the expiration date;
- vials with conjugate, calibrators and control (ready-to-use), sample diluent: at +2...+8 °C for 12 months; vials with calibrators and control (reconstituted): at +2...+8 °C for no more than 1 month after opening;
- vial with substrate: at +2...+8 °C until the expiration date, protected from light;
- vials with concentrated wash solution and stop solution: at +2...8 °C until the expiration date;
- wash solution prepared for use: at room temperature (+18...+25 °C) for no more than 5 days or at +2...+8°C for no more 4 weeks, in a firmly closed bottle.

Damaged Test Kits

In case of any severe damage of the test kit or components, it has to be informed in writing, during one week after receiving the kit. Usage of severely damaged single components for a test run is not recommended.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipaemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at +2...+8 °C for no more than 2 days. Aliquot and freeze samples for longer storage (-20 °C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

Serum samples collected from apparently healthy people (both males and females) at the age 21–45, were assayed with **SHBG kit**. The results are listed below. These limits should be considered as guidelines only.

Study Group	SHBG concentration range (nmol/L)	Median (nmol/L)
Healthy men (n =70)	12.4 – 78.4	38.5
Healthy non-pregnant women (n = 85)	14.1 – 129	67

It is highly recommended laboratory to determine its own reference range of SHBG concentrations.

7. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results.

8. REAGENT PREPARATION

Allow all the reagents to reach room temperature (+18...+25 °C), and then thoroughly stir.

MP Keep **microplate** at room temperature for at least 30 minutes before opening the bag. Place required number of strips onto strip holder. Place unused strips into the resealable zipper bag and reseal duly.

CAL CONTROL Calibrators and Control

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH P Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH P 20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature. Stir samples gently in order to ensure homogeneity.

Before the analysis all the serum samples (except calibrators and control) should be diluted 20-fold with sample diluent. If expected SHBG concentration in the serum sample is higher than in calibrator 5, the sample should be additionally diluted 20-fold with sample diluent:

Sample 1 (20-fold dilution): 380 μ L of sample diluent **DIL** + 20 μ L of serum sample.

Sample 2 (additional 20-fold dilution): 380 μ L of sample diluent **DIL** + 20 μ L of Sample 1.

Mix thoroughly after each dilution.

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration - Traceability

SHBG kit was calibrated against 1st WHO International Standard 95/560.

10.2. Specificity

According to Product specification provided by the supplier, both monoclonal antibodies used in the assay do not show cross-interaction with human serum albumin (HSA). Anti-SHBG monoclonal antibodies conjugated with HRP also do not show cross-reaction with thyroxin-binding globulin (TBG), corticosteroid-binding globulin (transcortin), AFP and human IgG.

10.3. Analytical Sensitivity

Analytical sensitivity of **SHBG kit**, i.e. concentration that can be distinguished from zero calibrator, is 2.0 nmol/L. It is defined as mean OD of 10 replicates of calibrator 0 plus 2 SD.

10.4. Measurement Range

SHBG kit was validated for measurement of SHBG concentration within the concentration diapason (without dilution) of 2.0-200 nmol/L.

10.5. Hook Effect

For **SHBG kit high dose hook effect** was not detected for concentrations up to 10 000 nmol/L. **High dose hook effect** was determined by spiking calibrator 0 matrix with antigen.

10.6. Intra- and Inter-Assay Variation

For **intra-assay CV** determination 8 serum samples were assayed in 9 replicates each. The results are shown below.

Sample	Mean SHBG concentration, nmol/L	Intra-assay CV	
		SD	CV, %
HS 1	10.8	0.51	4.7
HS 2	22.7	0.81	3.6
HS 3	27.9	0.68	2.4
HS 4	30.7	0.81	2.6
HS 5	40.1	1.60	4.0
HS 6	56.8	1.60	2.8
HS 7	95.3	2.84	3.0
HS 8	150	7.4	4.9

For **inter-assay CV** determination 8 serum samples were assayed 3 times by different operators with 1-week interval. Each specimen was assayed in 9 replicates. The results are shown below.

Sample	Mean SHBG concentration, nmol/L			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV,%
HS 1	29.4	32.3	31.0	1.45	4.7
HS 2	46.6	49.4	50.9	2.18	4.5

Sample	Mean SHBG concentration, nmol/L			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV,%
HS 3	61.7	67.8	67.4	3.41	5.2
HS 4	68.9	76.1	77.1	4.47	6.0
HS 5	78.6	85.9	87.7	4.82	5.7
HS 6	88.7	96.1	98.4	5.07	5.4
HS 7	99.9	108	108	4.7	4.4
HS 8	153	165	160	6.0	3.8

10.7. Dilution Parallelism of Serum Samples

Serial dilutions of three human serum samples with predetermined SHBG concentration in sample diluent were assayed with **SHBG kit** with the following result:

Sample	Dilution	Measured concentration, nmol/L	Expected concentration, nmol/L	Measured /expected concentration ratio, %
1	20-fold diluted	144.0		
	1:2	67.5	72.0	94
	1:4	34.3	36.0	95
	1:8	18.6	18.0	103
	1:16	9.35	9.0	104
2	20-fold diluted	102.0		
	1:2	47.3	51.0	93
	1:4	24.3	25.5	95
	1:8	12.2	12.8	96
	1:16	6.27	6.38	98
3	20-fold diluted	86.5		
	1:2	41.8	43.2	97

Sample	Dilution	Measured concentration, nmol/L	Expected concentration, nmol/L	Measured /expected concentration ratio, %
	1:4	22.8	21.6	105
	1:8	11.9	10.8	110
	1:16	6.18	5.4	114

11. LIMITATION OF THE METHOD

Clinical diagnoses should not be based on the results of in vitro diagnostic methods alone. To make a diagnosis, the physician should consider all available clinical and laboratory findings.

12. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.

- Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.

- Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.

- Do not use substrate, stop solution and wash solution supplied by other vendors.

- Use only "P"-labeled wash solution.


- Note that stop solution is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.


- Take into account the following common procedural notes:


- always pipette reagents into wells immediately after washing procedure;


- avoid contamination of the solutions;
 - in case of partial use of the kit, dispense only required volume of the reagent into the tray;
 - do not pour unused reagents back into the original vials;
 - avoid exposure to direct sunlight during incubations;
 - always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators and samples must not exceed 15 min;
 - the incubation temperature for all the immunological reactions must be kept at +37 °C;
 - do not touch the bottom of the wells;
 - calibrators should be measured in each separate assay.
- It is also recommended to measure each time SHBG concentration in the control.

- TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.

-  Source materials of human origin used for kit components preparation were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.

-  After usage strips, calibrators, control, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.

-  During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.

-  As the kit contains potentially hazardous material, the following precautions should be taken:

- do not smoke, eat or drink while performing the assay;
- always use protective gloves;
- never pipette material by mouth;
- in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.

- GLP including all general and individual regulations should be applied for the kit usage.



As the kit contains irritant (**CONJ**, **DIL**, **CAL**, **CONTROL**), the following precautions should be observed:

- P261 - Avoid breathing spray;
- P272 - Contaminated work clothing should not be allowed out of the workplace;
- P280 - Wear protective gloves/protective clothing/eye protection;
- P302+P352 - IF ON SKIN: Wash with plenty of soap and water;
- P333+P313 - If skin irritation or rash occurs: Get medical advice/attention;
- P363 - Wash contaminated clothing before reuse;
- P501 - Dispose of contents/container in accordance with national regulation.

Precautionary statements according to Regulation EC № 1272/2008.

13. MATERIALS PROVIDED

<p>MP</p>	<p>Microplate: 12 breakable 8-well strips (total 96 wells) coated with anti-SHBG monoclonal antibodies</p>	<p>1 pcs</p>
<p>CONJ</p>	<p>Conjugate: solution contains anti-SHBG monoclonal antibodies conjugated with HRP</p>	<p>14 mL, ready to use</p>
<p>0-5 CAL</p>	<p>SHBG calibrators: protein-based solution or lyophilized preparations containing known SHBG concentrations - 0; 5; 20; 50; 100; 200 nmol/L (approximate values). The concentrations of calibrators may be different for schemes with or without shaking.</p>	<p>6 vials, 0.5 mL each; ready to use or lyophilized preparations</p>
<p>CONTROL</p>	<p>SHBG control: protein-based solution or lyophilized preparation containing known SHBG concentration. The range of SHBG concentration may be different for schemes with or without shaking.</p>	<p>0.5 mL, ready to use or lyophilized preparation</p>
<p>SUB</p>	<p>Substrate (TMB solution): 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide</p>	<p>14 mL, ready to use</p>
<p>WASH P 20X</p>	<p>Wash solution P, 20X concentrated: surfactant in buffered saline, sufficient for preparation of 560 mL of solution</p>	<p>2x 14 mL, concentrated</p>
<p>STOP</p>	<p>Stop solution: 1 N HCl solution</p>	<p>14 mL,</p>

		ready to use
DIL	Sample diluent: protein-based solution with stabilizers	20 mL, ready to use

14. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- 1-channel calibrated variable precision pipettes, with disposable tips;
- 8-channel calibrated variable precision pipette, with disposable tips;
- microplate incubator (+37 °C) or microplate incubator/shaker (+37 °C, shaking speed 500–800 rpm);
- manual or automatic equipment for rinsing wells;
- calibrated microplate reader (450 nm);
- vortex tube mixer;
- deionized or distilled water;
- graduated beaker and cylinder of appropriate volume;
- latex or plastic gloves;
- trays for pipetting reagents with 8-channel pipette;
- disinfectant;
- absorbent material (for manual wash).

15. TEST PROCEDURE

SHBG kit is designed for 96 tests. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

15.1. Assay Procedure

15.1.1. Protocol with shaking

(see assay scheme, section 16)

A. Pipette:

- 20 μL of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates into the respective wells;

Leave wells A1-A2 empty for blank!

B. Pipette:

- 100 μL of conjugate **CONJ** into each well, except wells A1-A2.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for 1 hour at +37 °C while shaking (500–800 rpm).

D. Wash 5 times, as described in section 15.2.

E. Pipette 100 μL of substrate **SUB** into each well (including blank); incubate strips at room temperature (+18...+25 °C) in the dark for 15-30 minutes, depending on the colour intensity, or 10 minutes while shaking (500-800 rpm) at +37 °C.

F. Pipette 100 μL of stop solution **STOP** to all the wells in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at 450 nm within 20 min.

15.1.2. Protocol without shaking

(see assay scheme, section 17)

A. Pipette:

- **20 μ L** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates into the respective wells;

Leave wells A1-A2 empty for blank!

B. Pipette:

- **100 μ L** of conjugate **CONJ** into each well, **except wells A1-A2.**

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **2 hour at +37 °C** (pre-shake for 1-2 minutes at room temperature).

D. Wash 5 times, as described in section 15.2.

E. Pipette **100 μ L** of substrate **SUB** into each well (including blank); incubate strips **at room temperature (+18...+25 °C) in the dark for 15-30 minutes**, depending on the colour intensity.

F. Pipette **100 μ L** of stop solution **STOP** to all the wells in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within 20 min.**

15.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 5 wash cycles and a volume of 300 μ L of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually:

- remove the contents of the wells into a container with disinfectant;

- dispense 300 μ L of wash solution, prepared according to section 8.1, into each well, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 5 times;
strike the wells sharply on absorbent material to remove any liquid residue

15.3. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 5) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 5) calculated = $2.28 - 0.06 = 2.22$

15.3.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

- average blank OD (in wells A1-A2) ≤ 0.100 ;
- average OD of Cal 5 ≥ 1.0 (after blank subtraction);

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

15.3.2. Quantitative Determination

Specialized software for quantitative determination is recommended. Mean OD values of the calibrators at 450 nm versus their respective SHBG concentrations using 4PL or 5PL fit (see typical standard curve, fig. 2). Calculate concentration of SHBG in samples using standard curve.

Any extrapolation of the standard curve to SHBG concentration above the nominal value of calibrator 5 is forbidden. In this case the sample should be diluted 20-fold with sample diluent and re-tested. Multiply the measured concentration of additionally pre-diluted samples by additional dilution factor (20-fold).

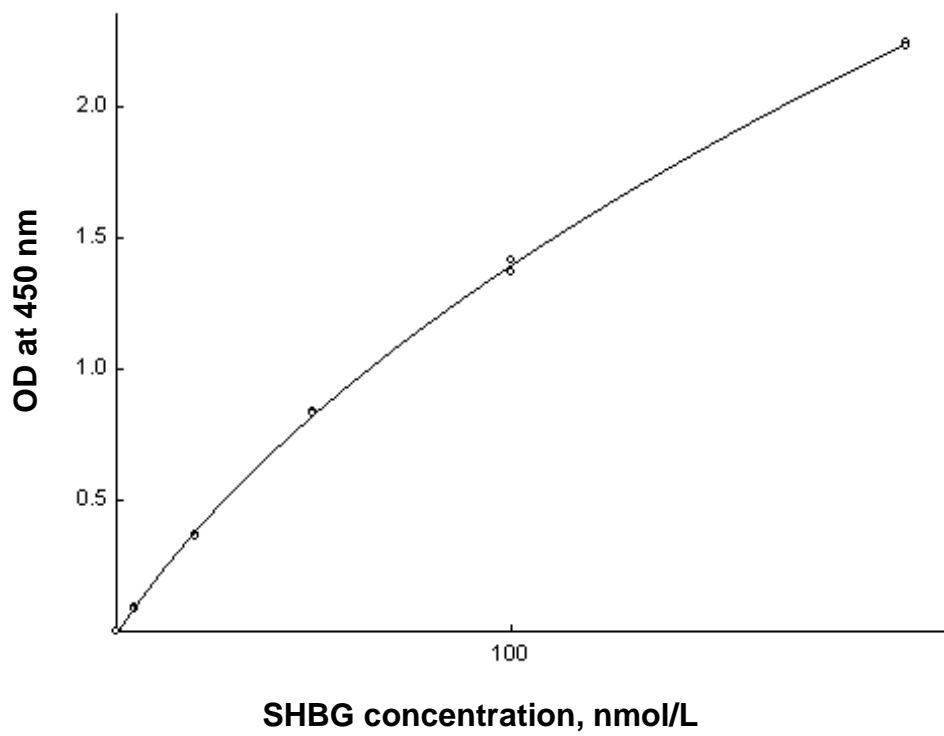


Fig. 2. Example of typical standard curve.
Do not use for evaluation of real assay data!

16. ASSAY SCHEME WITH SHAKING

Wells	«Blank»	CAL CONTROL	Samples
Reagents			
CAL CONTROL	–	20 µL	–
Samples	–	–	20 µL
CONJ	–	100 µL	100 µL
Incubation No.1	60 min, +37 °C, 500–800 rpm		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, +18...+25 °C, in the dark		
	10 min, +37 °C, 500–800 rpm		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18...+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

17. ASSAY SCHEME WITHOUT SHAKING

Wells	«Blank»	CAL CONTROL	Samples
Reagents			
CAL CONTROL	–	20 µL	–
Samples	–	–	20 µL
CONJ	–	100 µL	100 µL
Incubation No.1	120 min, +37 °C, (pre-shake for 1-2 minutes at room temperature).		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, +18...+25 °C, in the dark		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18...+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

BIOGENIX INC. PVT. LTD.

Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India

Email: biogenix2007@yahoo.com, info@biogenixinc.com

Website: www.biogenixinc.com

Customer care no: +919140971443