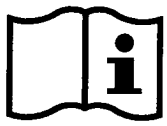
Product information

Information about other products is available at: www.demeditec.com

EN ISO 9001 certified company



Useŕs Manual

SHBG IRMA

The SHBG IRMA system provides a direct quantitative determination of SHBG in human serum.

IVD

CE

REF **DE46100**

Σ **100**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

Vers. ACE040501
/JS
Updated 120717

1. DESCRIPTION

The SHBG [^{125}I] IRMA system provides a direct quantitative determination of SHBG in human serum. SHBG can be assayed in the range of 0-250 nmol/l using 10 µl serum samples. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

2. INTRODUCTION

Sex hormone-binding globulin (SHBG), also known as Testosterone-estradiol binding globulin (TEBG) and sex steroid binding protein (SBP), is a circulating glycoprotein with a molecular weight of around 86000. It is thought to be synthesized in the liver, and in the circulation its biological function is the transport of sex steroid hormones. It has a high binding affinity for testosterone, dihydrotestosterone and estradiol.
Serum SHBG levels are relatively low at birth, increase to high levels during infancy, then decrease during puberty. The highest physiologic levels of SHBG are observed in pregnant, near-term maternal serum. Abnormal serum SHBG levels have been reported in number of conditions, including obesity and female hyperandrogenism (including polycystic ovary disease). Serum SHBG levels are inversely related to free, presumably bioactive, testosterone concentrations, a “free testosterone index” based on the ratio of total testosterone to SHBG has been characterized.

3. PRINCIPLE OF METHOD

The ^{125}I labelled signal-antibody binds to an epitope of the SHBG molecule, which is different from that recognised by the unlabelled capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a “sandwich”.
During a 2-hour incubation period with continuous agitation immune-complex is immobilised on the reactive surface of test tubes. Reaction mixture is then discarded, test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter.
The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of standards containing known amount of SHBG, the unknown concentration of SHBG in patient samples can determined.

4. CONTENTS OF THE KIT

1) 1 bottle TRACER, 32 ml, ready for use. Contains less than 740 kBq of 125I labelled anti-SHBG and biotin labelled anti-SHBG in buffer containing proteins, 0.1% sodium azide, red coloured.
2) 6 vials STANDARD(S0-S5), ready for use. 0.5ml, human serum containing 0.1% NaN3. Conc.: 0, 5, 15, 40, 100, 250 nmol/l.
3) 1 vial CONTROL SERUM, lyophilized 0.5 ml human serum, containing 0.1% NaN3. The concentration of control serum is specified in the quality certificate enclosed
4) 2 boxes COATED TUBES, ready for use. 2x50 plastic tube, coated with streptavidin.
5) 1 bottle WASH BUFFER CONCENTRATE, 20 ml, with 0.2% NaN3. Dilute with 700 ml distilled water before use.
6) Quality certificate
7) Pack leaflet

5. MATERIALS, TOOLS AND EQUIPMENT REQUIRED

- test tube rack
- precision pipettes with disposable tips (10µl, 300µl, 2ml)
- shaker
- plastic foil
- adsorbent tissue
- gamma counter

Recommended tools and equipment

- repeating pipettes
- dispenser with reservoir (instead of the 2-ml pipette)

6. SPECIMEN COLLECTION AND STORAGE

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 48 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Do not store serum samples longer than 4 months. Do not use lipemic, hemolyzed or turbid specimens. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided.

7. PREPARATION OF REAGENTS, STORAGE

Store the reagents between 2-8°C after opening. At this temperature reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.
Add 0.5 ml distilled water to the *lyophilised control serum*, and mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes.
Add the *wash buffer concentrate* to 700 ml distilled water. The diluted solution can be stored at 2-8 °C until expiry date of the kit.

CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

8. ASSAY PROCEDURE

(For a quick guide)

1. Label coated tubes in duplicate for each standard (S0-S5), control serum(C) and samples (P). Optionally, label two test tubes for total count (T).
2. Pipette **10 µl** each of STANDARD (S0-S5), CONTROL(C) and SAMPLES (P) into the properly labelled tubes.
3. Pipette **300 µl** of TRACER into each tube.
4. Gently vortex all tubes. Seal all tubes with a plastic foil. If optional total counts tubes are also prepared, place them separately from others.
5. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube. Incubate tubes for 2 hours at room temperature. *(Note: The efficient rotation is a critical factor to achieve good performance. An uneven or incomplete shaking may result in a serious error. Never use a rack type with open hole.)*
6. Add 2 ml diluted wash buffer to each tube and decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Repeat Step 6.
8. Count each tube for at least 60 seconds in a gamma counter.

9. CALCULATION OF RESULTS

Calculate the average CPM for each pair of assay tubes. Draw the standard curve by plotting mean CPM of each standard level (ordinate) against the respective concentration, except for 0 standard (abscissa) using log-log graph paper.
Obtain sample concentration by interpolation of sample counts on the standard curve.
For computerised calculations and/or quality assessment normalised specific binding values, rather than cpm values are used. Specific binding values can be calculated for each standard and sample according to the following equation:

$$B/T\% = S1\text{-}S5,C,P(cpm) - S0(cpm) / T(cpm) \times 100$$

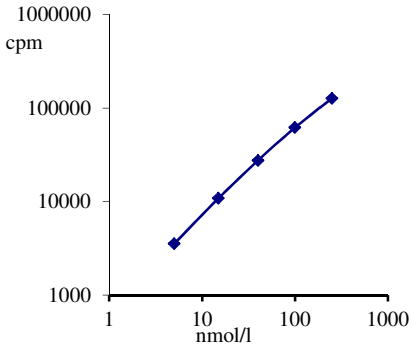
Assay Protocol, Pipetting Guide
(all volumes in microlitres)

	T	**S0-S5**	**C**	**P**
Standard		**10**		
Control			**10**	
Samples				**10**
Tracer	**300**	**300**	**300**	**300**
Vortex mix Rotate for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Typical assay data

Tubes	Count cpm	Mean cpm	Bo/T%	nmol/l
T	303150 294072	298611		
S0	45 40	42	0.014	
S1	3563 3489	3526	1.181	
S2	10799 10842	10821	3.624	
S3	27332 27780	27556	9.228	
S4	61341 62452	61896	20.73	
S5	126134 127507	126820	42.47	
C	29164 29522	29343		42.85

Typical standard curve



10. CHARACTERIZATION OF ASSAY

Calibration
Standards are calibrated against the WHO Standard, Code 95/560.

Assay parameters
NSB/T < 0.05%
B_{max}/T > 35 %

Analytical sensitivity
The analytical sensitivity is *0.11nmol/l*
It is defined as the concentration of SHBG equivalent to the mean CPM of 20 replicates of the zero standard.

Functional sensitivity
The value of functional sensitivity is found to be *0.22 nmol/l.*
It is defined as the value extrapolated to 20 % of the inter-assay imprecision profile obtained from 10 independent runs on patient samples with low endogenous SHBG concentration

Hook effect
There is no high dose “hook effect” up to a SHBG concentration of 835 nmol/l.

Specificity

Cross reactivity of the SHBG antiserum has been measured against human IgG (10 g/l) and human serum albumin (50 g/l). In both cases cross reactivity non-detectable.

Precision and reproducibility

Samples with 20 replicates in 1 assay run, and with duplicates in 12 runs were measured to determine intra-assay and inter-assay precision, respectively. Values obtained are shown below

Intra-assay		Inter-assay	
mean (nmol/l)	**CV %**	**Mean (nmol/l)**	**CV %**
3.11	5.42	0.91	4.97
7.48	5.42	3.11	4.10
28.47	4.99	6.62	3.35
77.20	4.91	26.83	6.04
129.32	7.00	43.33	4.14
207.39	8.58	72.47	3.83
		123.80	3.16
		192.79	4.56

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of SHBG. Values for 8 serum pools spiked with SHBG at 3 levels were as follows: *94.52 ± 5.21%*

Dilution test

4 samples were measured in a series of dilution (2, 4, 8, 16-fold) with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$$y = 0.9301x - 0.5552, \quad R=0.9962, \quad n=16$$

Expected values

It is recommended that each laboratory establish its own reference intervals.
In a population (n=134) of adult *female* blood donors the mean (±SD) serum concentration of SHBG was:

85±65 (range 10–330) nmol/l.

In a population (n=139) of adult *male* blood donors the mean (±SD) serum concentration of SHBG was:

32±12 (range 7.7–81) nmol/l.

Results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

11. ADDITIONAL INFORMATION

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

12. PRECAUTION

Radioactivity
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg).Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Chemical hazard
Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 75 mg.


Used by

Temperature limitation


Caution, consult accompanying documents


Biological risks


Consult instructions for use

In vitro diagnostic device


Manufacturer

REF Catalogue number


Radioactive material

Batch code

Control

Standard

Coated Tube

Tracer

Antiserum