



1. NAME AND INTENDED USE

SHBG-RIACT is a kit for the quantitative determination of human SHBG (Sex Hormone Binding Globulin), also called TeBG (Testosterone Estradiol Binding Globulin) or SBP (Sex Binding Protein) in serum or plasma.

2. INTRODUCTION

SHBG, like TBG (Thyroxin Binding Globulin) and CBG (Corticosteroid Binding Globulin) is one of the specific binding proteins in serum.

SHBG plays an important role in controlling the androgen-estrogen balance in plasma because:

- it specifically binds DHT and testosterone with very strong affinity (although 3 times weaker than DHT) and with lower affinity, estradiol;

- its concentration in plasma undergoes numerous hormonal regulations (estrogens, androgens, progestagens, thyroid hormones, cortisol...). Variations in the concentration of SBP in plasma adjust its bound and unbound estradiol and testosterone fractions.

Variations have been observed in the following physiopathological situations: hirsutism (↘) – acromegaly (↘) – estrogentherapy: treatment of menopause by substitution with natural or synthetic estrogens (↗) – oral contraceptives: (↗) with synthetic estrogens; (↘) with some synthetic progestogens – thyrotoxicosis with an autonomous nodule (↗) – cirrhosis (↗) – anorexia nervosa (↗) – obesity (↘)

3. PRINCIPLE

The SHBG is a solid phase "sandwich" immunoradiometric assay. Two monoclonal antibodies were prepared against two different antigenic sites of SHBG molecule. The first is coated on the solid phase (coated tube), and the second, radiolabeled with iodine 125, is used as a tracer.

SHBG molecules present in the standards or the samples to be tested are sandwiched between the two antibodies. Excess tracer is easily removed during the washing step of the procedure, and only the sandwich of coated antibody/antigen/tracer antibody remains on the tubes.

The amount of radioactivity bound to the tubes is thus proportional to the amount of SHBG present at the beginning of the assay.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTIT Y	STORAGE
COATED TUBES: ready –for use. Polystyrene tubes coated with anti-SHBG monoclonal mouse immunoglobulins.	2 packs of 50 tubes	2-8°C until the expiry date. Unused coated tubes removed from their pack must be stored in the plastic bag supplied with the kit.
Anti-SHBG 125I: liquid. Purified and 125I labelled anti-SHBG monoclonal mouse immunoglobulins : ≤ 185 KBq. Buffer, sodium azide 1g/l, bovine albumin, red dye.	1 32 ml vial	2-8°C until the expiry date.
STANDARDS: lyophilized*. 0, A,...E : buffer, bovine albumin, sodium merthiolate 0.1 g/l, human SHBG.	6 qs 0.5 ml vials	2-8°C until the expiry date. After reconstitution: Stable for 2 weeks at 2-8°C or until expiry date on kit at - 20°C.
CONTROLS 1 and 2: lyophilized**. Human serum, human SHBG, sodium merthiolate 0.1 g/l. Reconstitute the vials' contents with 0.5 ml of distilled water. Wait 5-10 minutes then mix.	2 qs 0.5 ml vials	2-8°C until the expiry date. After reconstitution: Stable for 2 weeks at 2-8°C or until expiry date on kit at - 20°C.
BUFFER: ready for use. This reagent is used as buffer or dilunt. Buffer, sodium azide 1g/l, bovine albumin.	1 55 ml vial	2-8°C until the expiry date.
WASHING SOLUTION: Sodium phosphate, NaCl, Tween 20, sodium merthiolate 0.7 g/l. Make up to 500 ml with distilled water. Shake.	1 70 ml vial	2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.
PLASTIC BAG	1	

(*) The true value of each standard or control is shown on its label.

(**) The acceptance range true values are printed on the vial label.



5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards. Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method for doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products. Sodium merthiolate is harmful via inhalation, skin contact or if swallowed (R20/21/22).

Danger of cumulative effects (R33).

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions. Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date. Do not mix reagents from different batches. Do not process more than 100 tubes at the same time. Avoid any microbic contamination of the reagents or of the water. Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum (if possible, collect blood in dry glass tubes) or non hemolysed plasma (do not use EDTA). If the test is to be carried out within 24 hours, serum and plasma must be stored at 2-8°C. Avoid successive freezing and thawing.

Dilutions

Should elevated SHBG levels be suspected, the diluent found in the kit is used for dilution. It is recommended to carry out the dilutions using disposable plastic tubes. Samples with titers higher than the concentration of the E standard are diluted 1/5 in the buffer.

7. ASSAY PROCEDURE

7.1. Equipment required

Precision micropipettes or similar, with disposable tips, capable of dispensing 10 µl and 300 µl (± 1%). Their calibration should be checked regularly. Distilled water.

Disposable plastic tubes. Vortex-type mixer. Circular horizontal shaker (400 rpm). Aspirating device or Pasteur pipette connected to a suction flask and vacuum pump or equivalent. Gamma scintillation counter calibrated for 125 iodine measurement.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagents into the tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:

Standard "0" group, for the determination of the non specific binding.

Standard groups, to establish the standard curve.

Control groups for controls.

Sx groups, to test serum or plasma samples.

It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.



Strictly respect the order in which reagents are to be added:

Add 10 µl of standards, controls or samples to the corresponding tubes.

Dispense 300 µl of buffer into each tube.

Mix gently each tube with a vortex-type mixer.

Incubate 30 minutes at room temperature (18-25°C) while continuing shaking at 360 rpm).

Wash the coated tubes as follows:

Aspirate the incubation medium carefully using the aspiration device.

Add 2.0 ml of diluted washing solution to each tube.

Finally, to avoid residual volume, carry out a thorough final aspiration.

To obtain reliable and reproducible results, the different washing steps have to be performed correctly: the addition of the washing solution must be carried out fast enough to create turbulence within the tubes.

Dispense 300 µl of anti-SHBG 125 I monoclonal antibody to all of the tubes.

Mix gently (Vortex).

Incubate 30 minutes at room temperature (18-25°C) under shaking (360 rpm).

Wash the coated tubes as previously described.

Measuring the remaining radioactivity bound to the tube with a gamma scintillation counter.

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background. Draw up the standard curve by plotting the standards' cpm against their concentrations. Read the sample values directly from the curve, correcting the read value for the dilution factor if necessary.

Typical standard curve (example only): this data must not be substituted for results obtained in the laboratory.

Tube group	Mean (cpm)	B/T	Concentration (nmol/l*)
Total activity	58 081	100	
Standard 0	69	0.1	0
Standard A	1 137	2.0	5
Standard B	3 967	6.8	20
Standard C	8 838	15.2	50
Standard D	16 057	27.7	100
Standard E	28 668	49.3	200
Control 1	5 187	8.9	27.1
Control 2	12 156	21.0	72.2

* The SHBG concentration expressed in nmol/l is determined referring to its binding capacity against 5α-dihydrotestosterone (DHT): 1 nmole of SHBG binds 1 nmole of DHT.

10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

11. EXPECTED VALUES

Each laboratory should establish its own reference ranges.

The data below gives an example of the serum values obtained with population of 141 presumed normal individuals.

	MEN			MENSTRUAL WOMEN		
	n	Mean ± SD (nmol/l)	Range	n	Mean ± SD (nmol/l)	Range
Site 1	23	29.7 ± 12.2	9 - 54	23	47.9 ± 12.9	30 - 69
Site 2	20	26.1 ± 10.6	12 - 46	15	62.1 ± 14.3	35 - 87
Site 3	30	27.5 ± 7.7	15 - 43	30	50.5 ± 16.6	18 - 83



12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 5 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 30 different series.

Sample	Within-run		Between-run	
	Mean (nmol/l)	CV%	Mean (nmol/l)	CV%
1	17.1	2.5	15.8	4.1
2	24.1	3.6	24.0	5.5
3	35.0	4.6	34.5	4.6
4	71.9	3.9	69.6	4.7
5	114.4	5.2	106.6	5.3

1.2. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being < 0.5 nmol/l.

ASSAY FLOW CHART

Tubes	Standards 0 to E Controls Samples µl	Buffer µl	Mix gently Incubate 30 minutes at 18-25°C under shaking (360 rpm) Aspirate Wash 1 time	Anti- SHBG 125l µl	Mix gently Incubate 30 minutes at 18-25°C under shaking (360 rpm) Aspirate Wash 1 time	Count
Standards	10	300		300		
Control	10	300		300		
Sample	10	300		300		