

SHBG

BL-40-CT

Bio-Line s.a.

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IMMUNORADIOMETRIC ASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN SEX HORMONE BINDING GLOBULIN IN HUMAN SERUM

100 Determinations

FOR IN VITRO DIAGNOSTIC USE ONLY

1. CLINICAL APPLICATIONS

Human Sex Hormone-Binding Globulin (**hSHBG**) is a circulating glycoprotein with a molecular weight of about 90000 (the most important role of SHBG is as a transport protein for estrogens and androgens in the peripheral circulation).

Level of Sex Hormone Binding Globulin is increased by estrogens, during pregnancy and by thyroid hormones. Level of SHBG is decreased by androgens (external or internal production) and some drugs.

SHBG measurement in serum is also a very important evolutivity index for non strictly steroid related diseases. SHBG levels correlate well with thyroid function and high values are found in cases of reduced liver function as in cirrhosis.

2. PRINCIPLE OF THE ASSAY

This test kit is an immunoradiometric assay (IRMA) based on coated-tubes with monoclonal antibodies directed against distinct epitopes of the molecule of SHBG.

Two capture antibodies are coated on the inner wall of tubes. SHBG of the calibrators or of the samples is captured by these antibodies.

Addition of the third antibody labeled with ¹²⁵Iodine completes the system, allowing the formation of a bridge between the coated antibodies and the labeled antibody.

After washing, the remaining radioactivity bound to the tubes is directly related to the concentration of SHBG in the calibrators or samples.

The careful choice of the three monoclonal antibodies allows high specificity and high sensitivity and avoids excess of specificity which is sometimes reproached to immunometric assays with only two monoclonals.

This test kit exists also under a form dedicated to R&D Pharmaceutical laboratories. This version (Q3) is available on special request only.

3. MATERIAL PROVIDED

- The reagents are sufficient for 100 determinations.
- Store the kit and reagents at 2-8°C.
- The expiration date of each reagent is shown on the label.

1. **Radioactive Tracer:** 1 vial of 22 ml anti-hSHBG labeled with ¹²⁵I, in phosphate buffer containing BSA. Radioactivity : ± 275 kBq. Preservatives : NaN₃ < 0.1%.
2. **Calibrators:** 7 vials containing 0.7 ml of hSHBG in horse serum, pre-diluted. Ready for use. Concentrations : 0 - 10 - 25 - 40 - 75 - 125 - 250 nmol/l. Preservative : NaN₃ < 0.1%.
3. **Control:** 1 vial containing 0.7 ml human serum prediluted. Ready for use. **For the exact values, refer to the values indicated on the quality control data sheet.** Preservative : NaN₃ < 0.1%.
4. **Coated Tubes:** 100 tubes coated with two mouse monoclonal antibodies directed against hSHBG. Unused tubes must be stored at 2-8°C, protected from moisture.
5. **Washing Solution (50 x concentrated):** 1 vial of 20 ml TRIS-HCl buffer with detergent Preservative : NaN₃ < 0.1%. Bring to 1000 ml with distilled water. The diluted washing solution is stable for 2 months at 2-8°C.
6. **Diluent:** 1 vial containing 100 ml phosphate buffer solution with BSA Preservative : NaN₃ < 0.1%. Ready for use.

ADDITIONAL MATERIAL PROVIDED FOR Q3 VERSION.

1. **Control Serum:** 1 set of additional 2 control vials containing each 0.7 ml human serum prediluted. Ready for use. **For the exact values, refer to the values indicated on the quality control data sheet.** Preservative : NaN₃ < 0.1%.
2. **High value sample diluent :** 1 vial containing 3 ml of diluent. Preservative: NaN₃ < 0.1%. If high values are expected, the original sample should be diluted with this diluent.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Plastic test tubes
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Graduated cylinder.
- Aspiration pump or automated washing device.
- Scintillation gamma counter.
- Distilled water.
- Orbital shaker adjustable at 150 rpm.

5. WARNINGS AND PRECAUTIONS

In order to obtain reproducible results, the following rules must be observed :

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; to this purpose disposable tips should be used for each sample and reagent.

In order to avoid personal and environmental contamination, the following precautions must be observed :

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit has been tested and found negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious; therefore, the assay waste must be decontaminated and disposed of, in accordance with established safety procedures. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
- Acquisition, storage, use and disposal of radioactive material (liquid and solid) are subject to regulation and ordination of local authorities.

6. SPECIMEN COLLECTION

Only serum sample may be used. Highly lipemic or hemolyzed samples must be discarded. Keep samples at 2-8°C for 1 day; for longer periods it is advisable to freeze samples in aliquots at -20°C. Repeated freezing and thawing of samples should be avoided. Before analysis, samples must be diluted 1/51 in the SHBG diluent as follow :

Sample 20 µl + SHBG Diluent 1 ml

Do not attempt to dilute the calibrators and control serum. They are already pre-diluted and ready for use.

7. ASSAY PROCEDURE

- Bring all reagents and samples to room temperature prior to use.
- Before use, mix the samples by inversion.
- For all calibrators, a duplicated measure is recommended.

1. Prepare plain tubes for Total Counts, and coated tubes for Calibrators, Samples and Control.
2. Pipette 50 µl of Calibrators, Control Serum and pre-diluted Samples into the appropriate coated tubes. Pipette directly to the bottom of the tubes.
3. Add 200 µl radioactive tracer (¹²⁵I labeled anti-hSHBG) to all tubes. Mix gently the tubes on vortex and incubate for 90 minutes on an orbital shaker set at 150 rpm.
4. Aspirate the contents of each tube, except the tubes for Total Counts.
5. Add 2 ml of diluted washing solution to every tube, except tubes for Total Counts and aspirate thoroughly or decant the contents of all tubes on absorbent paper.
6. Count the radioactivity bound to the tubes for 1 minute in a gamma counter. We suggest to control the background of the instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background must be reduced to a minimum or adjusted properly.

SCHEME OF THE ASSAY

Tubes	Total Activity	Calibrators	Control	Samples
Reagent				
Calibrators	----	50 µl	----	----
Control	----	----	50 µl	----
Samples	----	----	----	50 µl
Tracer	200 µl	200 µl	200 µl	200 µl

- Incubate: 90 min R.T. shaking (150 rpm)
 - Aspirate and wash: 1 x 2 ml
 - Count

8. CALCULATION OF THE RESULTS

Draw the calibration curve on log/lin graph by plotting the B/T (%) obtained for each calibrator (y-axis) against the relative concentration (x-axis). Calculate the B/T (%) of each sample and read the concentration by interpolating on the calibration curve.

EXAMPLE OF CALCULATION

The values reported below must be considered as an example and may not be used in place of experimental data

Description	Average cpm.	B/T (%)	SHBG (nmol/l)
Total Activity (T)	109800	-	-
CAL 0	140	0.1	0
CAL 1	3572	3.3	10
CAL 2	10255	9.3	25
CAL 3	15037	13.7	40
CAL 4	28925	26.3	75
CAL 5	44924	40.9	125
CAL 6	64577	58.8	250
CONTROL	28855	26.3	74.8
P1	22839	20.8	59.2
P2	45754	41.7	133.0

9. REFERENCE VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

	SHBG (nmol/l) range	N
Women	20 - 85	49
Men	9 - 55	50

Children : high concentration of SHBG dropping over the pre-pubertal, pubertal and adolescent periods to adult levels.

Aging adults : the SHBG level has been reported to increase with age in adults.

Pregnancy : during pregnancy, SHBG level increases considerably, up to 200 – 400 nmol/l during the 3rd trimester⁴.

Cirrhosis of the liver : increased

SHBG level varies in various physiopathological states :

- SHBG is increased by synthetic estrogens depending on the dose and type of estrogen³.
- SHBG has been reported to be lowered by levonorgestrel⁶.

The concentration of SHBG can be used to calculate the "Free Androgen Index" (FAI) :

$$FAI = \frac{\text{Total Testosterone (nmol/l)}}{\text{SHBG (nmol/l)}} \times 100$$

The "Free Androgen Index" is a very useful parameter to evaluate hyperandrogenism in women^{5,11}.

SHBG can also be used to calculate Free Testosterone and Free Estradiol concentrations according to the law of mass action¹².

10. PERFORMANCE OF THE ASSAY

SENSITIVITY

Analytical sensitivity

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value + 2 S.D.). This dose is 0.26 nmol/l.

Functional sensitivity

The functional assay sensitivity is the lowest value which is measured with a precision of maximum 20% inter-assay variance. For the SHBG, this value is lower than 0.3 nmol/l.

PRECISION

Precision was evaluated upon intra- and inter-assay variability, at different analyte concentrations.

Intra-assay

Serum	Mean	±	S.D.	C.V. (%)	N
1	15.1	±	0.5	3.3	20
2	44.7	±	1.3	2.9	20
3	151.5	±	7.9	5.2	20

Inter-assay

Serum	Mean	±	S.D.	C.V. (%)	N
1	15.5	±	0.5	2.9	9
2	45.1	±	2.1	4.6	9
3	163.3	±	9.4	5.8	9

ACCURACY

The accuracy of the method has been evaluated by recovery and parallelism tests.

11. BIBLIOGRAPHIE – BIBLIOGRAPHY – ΒΙΒΛΙΟΓΡΑΦΙΑ – BIBLIOGRAFIE – BIBLIOGRAFIA - BIBLIOGRAFÍA

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