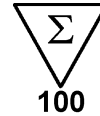


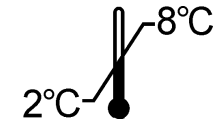


# SHBG

REF **SHBG-RIACT**



IVD



<p><b>Trousse permettant de déterminer la concentration en SHBG (Sex Hormone Binding Globuline) humaine, nommée également TeBG (Testostérone Estradiol Binding Globulin) ou SBP (Sex Binding Protein) dans le sérum ou le plasma</b> Pour diagnostic In Vitro</p> <p>La trousse contient :</p> <table border="0"> <tr> <td> Tubes revêtus</td> <td>2 x 50</td> </tr> <tr> <td> Traceur ≤ <b>185 kBq</b></td> <td>1 x 32 mL</td> </tr> <tr> <td> Calibrateur 0</td> <td>1 x 0,5 mL</td> </tr> <tr> <td> Calibrateurs A – E</td> <td>5 x 0,5 mL</td> </tr> <tr> <td> Contrôles</td> <td>2 x 0,5 mL</td> </tr> <tr> <td> Tampon</td> <td>1 x 55 mL</td> </tr> <tr> <td> Réactif de lavage (H330/H373/H411)</td> <td>1 x 70 mL</td> </tr> <tr> <td> Sachet plastique</td> <td>1</td> </tr> <tr> <td> Notice d'utilisation</td> <td>1</td> </tr> </table> <p><b>Attention :</b> Certains réactifs contiennent de l'azoture de sodium</p>	Tubes revêtus	2 x 50	Traceur ≤ <b>185 kBq</b>	1 x 32 mL	Calibrateur 0	1 x 0,5 mL	Calibrateurs A – E	5 x 0,5 mL	Contrôles	2 x 0,5 mL	Tampon	1 x 55 mL	Réactif de lavage (H330/H373/H411)	1 x 70 mL	Sachet plastique	1	Notice d'utilisation	1	<p><b>Kit for the quantitative determination of SHBG (Sex Hormone Binding Globulin) human, also called TeBG (Testosterone Estradiol Binding Globulin) or SBP (Sex Binding Protein) in serum or plasma</b> <b>For In Vitro diagnostic use</b></p> <p>Kit content :</p> <table border="0"> <tr> <td> Coated tubes</td> <td>2 x 50</td> </tr> <tr> <td> Tracer ≤ <b>185 kBq</b></td> <td>1 x 32 mL</td> </tr> <tr> <td> Calibrator 0</td> <td>1 x 0.5 mL</td> </tr> <tr> <td> Calibrators A – E</td> <td>5 x 0.5 mL</td> </tr> <tr> <td> Control serum</td> <td>2 x 0.5 mL</td> </tr> <tr> <td> Buffer</td> <td>1 x 55 mL</td> </tr> <tr> <td> Wash reagent (H330/H373/H411)</td> <td>1 x 70 mL</td> </tr> <tr> <td> Plastic bag</td> <td>1</td> </tr> <tr> <td> Instruction for use</td> <td>1</td> </tr> </table> <p><b>Warning :</b> Some reagents contain sodium azide</p>	Coated tubes	2 x 50	Tracer ≤ <b>185 kBq</b>	1 x 32 mL	Calibrator 0	1 x 0.5 mL	Calibrators A – E	5 x 0.5 mL	Control serum	2 x 0.5 mL	Buffer	1 x 55 mL	Wash reagent (H330/H373/H411)	1 x 70 mL	Plastic bag	1	Instruction for use	1
Tubes revêtus	2 x 50																																				
Traceur ≤ <b>185 kBq</b>	1 x 32 mL																																				
Calibrateur 0	1 x 0,5 mL																																				
Calibrateurs A – E	5 x 0,5 mL																																				
Contrôles	2 x 0,5 mL																																				
Tampon	1 x 55 mL																																				
Réactif de lavage (H330/H373/H411)	1 x 70 mL																																				
Sachet plastique	1																																				
Notice d'utilisation	1																																				
Coated tubes	2 x 50																																				
Tracer ≤ <b>185 kBq</b>	1 x 32 mL																																				
Calibrator 0	1 x 0.5 mL																																				
Calibrators A – E	5 x 0.5 mL																																				
Control serum	2 x 0.5 mL																																				
Buffer	1 x 55 mL																																				
Wash reagent (H330/H373/H411)	1 x 70 mL																																				
Plastic bag	1																																				
Instruction for use	1																																				
<p><b>Kit zur Bestimmung der SHBG-Konzentration (Sexualhormonbindendes Globulin), auch TeBG (Testosteron-Estradiol-bindendes Globulin) oder SBP (Sex Binding Protein) genannt, in Serum oder Plasma</b> <b>Zur In Vitro Diagnostik</b></p> <p>Inhalt des Kits :</p> <table border="0"> <tr> <td> Teströhrchen beschichtet</td> <td>2 x 50</td> </tr> <tr> <td> Tracer ≤ <b>185 kBq</b></td> <td>1 x 32 mL</td> </tr> <tr> <td> Kalibrator 0</td> <td>1 x 0,5 mL</td> </tr> <tr> <td> Kalibratoren A – E</td> <td>5 x 0,5 mL</td> </tr> <tr> <td> Kontrolle</td> <td>2 x 0,5 mL</td> </tr> <tr> <td> Puffer</td> <td>1 x 55 mL</td> </tr> <tr> <td> Waschlösung (H330/H373/H411)</td> <td>1 x 70 mL</td> </tr> <tr> <td> Plastikbeutel</td> <td>1</td> </tr> <tr> <td> Gebrauchsinformation</td> <td>1</td> </tr> </table> <p><b>Achtung :</b> Einige Reagenzien enthalten Natriumazid</p>	Teströhrchen beschichtet	2 x 50	Tracer ≤ <b>185 kBq</b>	1 x 32 mL	Kalibrator 0	1 x 0,5 mL	Kalibratoren A – E	5 x 0,5 mL	Kontrolle	2 x 0,5 mL	Puffer	1 x 55 mL	Waschlösung (H330/H373/H411)	1 x 70 mL	Plastikbeutel	1	Gebrauchsinformation	1	<p><b>kit per la determinazione della concentrazione di SHBG (Sex Hormone Binding Globulin) umana, definita anche TeBG (Testosterone Estradiol Binding Globulin) o SBP (Sex Binding Protein)</b> <b>Per uso diagnostico In Vitro</b></p> <p>Contenuto del kit :</p> <table border="0"> <tr> <td> Provette coattate</td> <td>2 x 50</td> </tr> <tr> <td> Tracciante ≤ <b>185 kBq</b></td> <td>1 x 32 mL</td> </tr> <tr> <td> Calibratore 0</td> <td>1 x 0,5 mL</td> </tr> <tr> <td> Calibratori A – E</td> <td>5 x 0,5 mL</td> </tr> <tr> <td> Siero di controllo</td> <td>2 x 0,5 mL</td> </tr> <tr> <td> Tampone</td> <td>1 x 55 mL</td> </tr> <tr> <td> Reagente di lavaggio (H330/H373/H411)</td> <td>1 x 70 mL</td> </tr> <tr> <td> Sacchetto di plastica</td> <td>1</td> </tr> <tr> <td> Istruzioni per l'uso</td> <td>1</td> </tr> </table> <p><b>Attenzione :</b> Alcuni reagenti contengono sodio azide</p>	Provette coattate	2 x 50	Tracciante ≤ <b>185 kBq</b>	1 x 32 mL	Calibratore 0	1 x 0,5 mL	Calibratori A – E	5 x 0,5 mL	Siero di controllo	2 x 0,5 mL	Tampone	1 x 55 mL	Reagente di lavaggio (H330/H373/H411)	1 x 70 mL	Sacchetto di plastica	1	Istruzioni per l'uso	1
Teströhrchen beschichtet	2 x 50																																				
Tracer ≤ <b>185 kBq</b>	1 x 32 mL																																				
Kalibrator 0	1 x 0,5 mL																																				
Kalibratoren A – E	5 x 0,5 mL																																				
Kontrolle	2 x 0,5 mL																																				
Puffer	1 x 55 mL																																				
Waschlösung (H330/H373/H411)	1 x 70 mL																																				
Plastikbeutel	1																																				
Gebrauchsinformation	1																																				
Provette coattate	2 x 50																																				
Tracciante ≤ <b>185 kBq</b>	1 x 32 mL																																				
Calibratore 0	1 x 0,5 mL																																				
Calibratori A – E	5 x 0,5 mL																																				
Siero di controllo	2 x 0,5 mL																																				
Tampone	1 x 55 mL																																				
Reagente di lavaggio (H330/H373/H411)	1 x 70 mL																																				
Sacchetto di plastica	1																																				
Istruzioni per l'uso	1																																				

FRA
















ENG

DEU

ITA

POL

SRP

	Explication des symboles	Explanation of symbols	Erläuterung der Symbole	Spiegazione dei simboli	Wyjaśnienie symboli	Objašnjenje simbola
	Conforme aux normes européennes	European conformity	CE-Konformitätskennzeichnung	Conformita europea	Zgodne z normami europejskimi	Evropska usaglašenost
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Graniczna temperatura przechowywania	Ograničenje temperature za čuvanje
	N° de lot	Batch code	Chargencode	codice lotto	Numer partii	Šifra serije
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Zużyć do	Upotrebiti do
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Patrz dołączona ulotka	Pogledajte uputstvo za upotrebu
	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Diagnostyka In Vitro	Uređaj za dijagnostiku <i>in vitro</i>
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Wyprodukowane przez	Proizveo
	Référence	Catalogue number	Katalog Nr.	N. catalogo	Wzorzec	Kataloški broj
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Liczba probówek	Broj određivanja
	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Probówki powlekane	Obložene epruvete
	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Traccianti radioattivo	Znacznik radioaktywny	Radioaktivni indikator
	Calibrateur	Calibrator	Kalibrator	Calibratore	Kalibrator	Kalibrator
	Contrôle	Control	Kontrolle	Controllo	Kontrola	Kontrola
	Tampon	Buffer	Puffer	Tampone	Bufor	Pufer
	Solution de lavage	Wash solution	Waschlotion	Soluzione di lavaggio	Roztwór płuczący	Rastvor za pranje

**FRA** Modifications par rapport à la version précédente :  
Nouveau logo

**ENG** Changes from the previous version:  
New logo

**DEU** Änderungen gegenüber der Vorgängerversion:  
Neues Logo

**ITA** Modifiche rispetto alla versione precedente:  
Nuovo logo

**POL** Zmiany w stosunku do poprzedniej wersji:  
Nowe logo

**SRP** Promene od prethodne verzije:  
Novi logotip

**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.

The kit is intended for professional use.

**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 (♂) – estrogen therapy: 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 calibrators 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	SYMBOLS	QUANTITY	STORAGE
<b>COATED TUBES:</b> ready for use. Polystyrene tubes coated with anti-SHBG monoclonal mouse immunoglobulins.	CT	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.
<b>Anti-SHBG 125I:</b> liquid. Purified and 125I labelled anti-SHBG monoclonal mouse immunoglobulins : ≤ 185 KBq. Buffer, sodium azide 1g/L, bovine albumin, red dye.	TRACER	1 32 mL vial	2-8°C until the expiry date.
<b>CALIBRATORS:</b> lyophilized*. 0, 5, 20, 50, 100, 180 nmol/L: buffer, bovine albumin, sodium merthiolate 0.1 g/L, human SHBG.	CAL	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.
<b>CONTROLS 1 and 2:</b> 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.	CONTROL	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.
<b>BUFFER:</b> ready for use. This reagent is used as buffer or diluent. Buffer, sodium azide 1g/L, bovine albumin.	BUF	1 55 mL vial	2-8°C until the expiry date.
<b>WASHING SOLUTION:</b> Sodium phosphate, NaCl, Tween 20, sodium merthiolate 0.7 g/L. Make up to 500 ml with distilled water. Shake.	BUF RCNS	1 70 mL vial	2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.
<b>PLASTIC BAG</b>		1	

(\*) The true value of each calibrator 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 calibrator 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. 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:

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

Calibrator groups, to establish the calibrator curve.

Control groups for controls.

Sx groups, to test serum or plasma samples.

It is recommended that the assay be performed in duplicate for the calibrators, the controls and the samples.

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

Add 10 µL of calibrators, 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

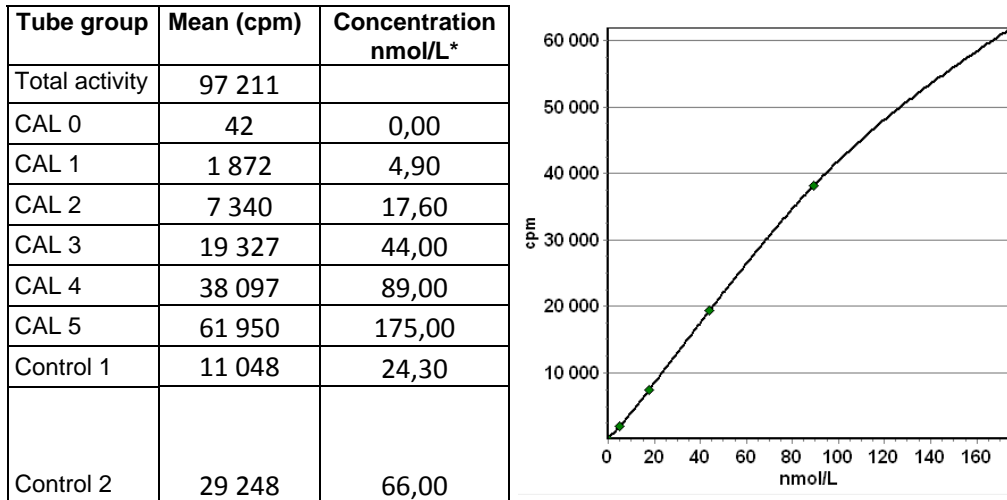
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

## 9. RESULTS

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

The spline mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

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



\* The SHBG concentration expressed in nmol/L is determined referring to its binding capacity against 5 $\alpha$ -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 calibrator. 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 $\pm$ SD (nmol/L)	Range	n	Mean $\pm$ SD (nmol/L)	Range
Site 1	23	29.7 $\pm$ 12.2	9 - 54	23	47.9 $\pm$ 12.9	30 - 69
Site 2	20	26.1 $\pm$ 10.6	12 - 46	15	62.1 $\pm$ 14.3	35 - 87
Site 3	30	27.5 $\pm$ 7.7	15 - 43	30	50.5 $\pm$ 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

### 12.2. 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.

### 12.3 Interference

No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L, and 20 g/L, has been observed.

The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector to the tracer (non-specific mouse immunoglobulins). However, we cannot guarantee that this protection is exhaustive.

### ASSAY FLOW CHART

Tubes	Calibrators 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 125I μL	Mix gently Incubate 30 minutes at 18-25°C under shaking (360 rpm) Aspirate Wash 1 time	Count
Calibrators	10	300		300		
Control	10	300		300		
Sample	10	300		300		
				300		

## **Bibliography**

Barbe P, Bennet A, Stebenet M, Perret B and Louvet JP. Sex-hormone-binding globulin and protein-energy malnutrition indexes as indicators of nutritional status in women with anorexia nervosa. *Am J Clin Nutr.* 1993;57:319-22.

Forest MG, Pugeat M. Binding Protein of steroid hormones. Colloque INSERM. John Libbey Eurotext. London and Paris. 1986;149.

Lindstedt G, Lunberg PA, Lapidus L, Lundgren H, Bengtsson C, Björntorp P. Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM: 12 yr follow-up of population study of women in Gothenburg. *Diabetes.* 1990;40:123-8.

Murphy S, Khaw K, Cassidy A and Compston JE. Sex hormones and bone mineral density in elderly men. *Bone and Mineral.* 1993;20:133-40.

Pugeat M, Garrel D, Estour B, Lejeune H, Kurzer M, Tourniaire J et Forest mg. Sex steroid binding protein in nonendocrine diseases. *Annals New York Academy of Sciences.* 1988;235-47.

Pugeat MM, Crave JC, Tourniaire J, Forest MG. Clinical utility of sex hormone-binding globulin measurement. *Horm Res.* 1996;45:148-55.

Rosner W. Errors in the measurement of plasma free testosterone. *J Clin Endocrinol Metab.* 1997;82:2014-5.