# Elecsys SHBG

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| REF | | Σ | SYSTEM |
|---|---|---|---|
| 07258496190 | 07258496500 | 300 | **cobas e** 402<br>**cobas e** 801 |

**English**

**System information**

| Short name | ACN (application code number) |
|---|---|
| SHBG | 10071 |

**Intended use**

Immunoassay for the in vitro quantitative determination of sex hormone-binding globulin in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

**Summary**

Sex hormone-binding globulin (SHBG) is the blood transport protein for testosterone and estradiol (E2). It is a large glycoprotein with a molecular weight of about 95 kDa, and exists as a homodimer composed of two identical subunits. Each subunit contains two disulfide bridges.[1,2] Generally, optimal steroid binding to SHBG will require a planar C19 steroid with a 17α-OH group, and an electronegative functional group at C3, whereas dissociated subunits of SHBG do not bind steroid and the two subunits associate to form a single steroid-binding site.[3]

The level of SHBG binding affinity for steroids from high to low is as following: dihydrotestosterone, testosterone, androstenediol, E2 and estrone. SHBG also weakly binds with dehydroepiandrosterone (DHEA) however not with dehydroepiandrosterone sulfate (DHEA-S).[4]

SHBG is produced mainly by the liver and its synthesis and secretion are regulated by estrogen and negatively influenced by liver fat content and inflammatory cytokines.[4,5,6,7,8,9] Decreased SHBG serum levels are associated with conditions where elevated androgen levels are present or where the effect of androgen on its target organs is excessive. This explains the gender-related differences seen between men and women, especially during puberty. However, decreased SHBG levels are also seen in inflammation and in case of a diet leading to fat build-up in the liver e.g. rich in monosaccharides, particularly fructose. Low serum SHBG concentrations may correlate with cardiovascular disease risk,[10,11] type 2 diabetes,[12,13] as well as breast cancer.[14] High serum SHBG concentrations have been proposed to be associated with change of diet resulting in loss of weight. Moreover, there is increasing evidence that plasma SHBG levels could serve as a biomarker for diseases with chronic inflammation and as indicator of response to anti-inflammatory treatment.[6]

Low SHBG titer can be an important indicator of an excessive/chronic androgenic action where androgen levels are normal, but where clinical symptoms would seem to indicate androgen in excess.[15]

By calculating the free androgen index (FAI), also called free testosterone index (FTI), from the ratio of total testosterone (T) to SHBG [% FAI or FTI = (100 T/SHBG)], it is possible to calculate the approximate amount of free testosterone, as there is a direct correlation between FAI and FT. By additionally taking the non-specifically albumin-bound testosterone into account, it is possible to calculate the bioavailable testosterone, which is the sum of free testosterone and the albumin-bound testosterone fraction, calculated via the association constant to albumin.[16] A similar calculation can be made for E2.[17] Only free testosterone is biologically active, and it best indicates the clinical situation of the patient. Free and bioavailable testosterone are also referred to as non-SHBG-bound testosterone and can be obtained by precipitation of the SHBG-bound-testosterone with ammonium sulfate, and by equilibrium dialysis.[18]

Elevated SHBG levels can be seen in elderly men, and are often found in patients with hyperthyroidism and cirrhosis. SHBG levels also increase when oral contraceptives or antiepileptic drugs are taken. Pregnant women have markedly higher SHBG serum concentrations due to their increased estrogen production. Decreased SHBG concentrations are often seen in patients with hypothyroidism, polycystic ovarian syndrome, obesity, hirsutism, elevated androgen levels, alopecia, and acromegaly. The Elecsys SHBG assay employs two monoclonal antibodies specifically directed against human SHBG.

Cross-reactivity with $\alpha_1$-fetoprotein (AFP), corticosteroid binding globulin (CBG), DHT, E2, fibrinogen, human immunoglobulin A (IgA), human immunoglobulin G (IgG), plasminogen, thyroxine binding globulin (TBG), testosterone, thyroglobulin (Tg), transferrin, and thyrotropin (TSH) is negligible.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample, a biotinylated monoclonal SHBG-specific antibody, and a monoclonal SHBG-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

**Reagents - working solutions**

The **cobas e** pack is labeled as SHBG.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-SHBG-Ab~biotin, 1 bottle, 21 mL:
Biotinylated monoclonal anti-SHBG antibody (mouse) 1.25 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-SHBG-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 21 mL:
Monoclonal anti-SHBG antibody (mouse) labeled with ruthenium complex 1.25 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

**Precautions and warnings**

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

**Prevention:**

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

**Response:**

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

**Disposal:**

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

**Reagent handling**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

**Storage and stability**

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

| Stability: | |
|---|---|
| unopened at 2-8 °C | up to the stated expiration date |
| on the analyzers | 16 weeks |

**Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel, or lithium heparin plasma.

Li-heparin plasma tubes containing separating gel can be used.

Do not use EDTA plasma.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 7 days at 2-8 °C, 12 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**Materials required (but not provided)**

- REF 03052028190, SHBG CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 07299010190, Diluent MultiAssay, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

**Calibration**

Traceability: This method has been standardized against the 1st International Standard for SHBG from the National Institute for Biological Standards and Control (NIBSC) code 95/560.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

**Quality control**

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

The analyzer automatically calculates the analyte concentration of each sample either in nmol/L, µg/mL or mg/L.

Conversion factors: nmol/L x 0.095 = µg/mL (mg/L)

µg/mL (mg/L) x 10.53 = nmol/L

**Limitations - interference**

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

*Endogenous substances*

| Compound | Concentration tested |
|---|---|
| Bilirubin | ≤ 1129 µmol/L or ≤ 66 mg/dL |
| Hemoglobin | ≤ 0.62 mmol/L or ≤ 1000 mg/dL |
| Intralipid | ≤ 2700 mg/dL |

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| Compound | Concentration tested |
|---|---|
| Biotin | ≤ 287 nmol/L or ≤ 70 ng/mL |
| Rheumatoid factors | ≤ 1200 IU/mL |
| Human serum albumin | ≤ 7 g/dL |

Criterion: For concentrations of 0.8-20 nmol/L the deviation is ≤ 2 nmol/L. For concentrations > 20 nmol/L the deviation is ≤ 10 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at SHBG concentrations up to 1000 nmol/L.

*Pharmaceutical substances*
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**Limits and ranges**

**Measuring range**

0.8-200 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.8 nmol/L. Values above the measuring range are reported as > 200 nmol/L (or up to 2000 nmol/L for 10-fold diluted samples).

**Lower limits of measurement**

*Limit of Blank, Limit of Detection and Limit of Quantitation*

Limit of Blank = 0.5 nmol/L

Limit of Detection = 0.8 nmol/L

Limit of Quantitation = 2 nmol/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95$^{th}$ percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

**Dilution**

Samples with SHBG concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 20 nmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

**Expected values**

The following table shows the results obtained from a group of 415 males and 343 females using the Elecsys SHBG assay. All subjects were apparently healthy, non-obese (BMI, body mass index ≤ 30), non-pregnant adults without intake of any contraceptive or relevant prescription drugs (study number CIM 000669). Blood samples were taken from fasting donors between 6.30 am and 2.00 pm. This clinical study focusing on the Elecsys Testosterone II assay included measurements in parallel using the Elecsys SHBG assay. Please refer to the Elecsys Testosterone II package insert for SHBG values in combination with testosterone.

**SHBG**

| | | SHBG (nmol/L) | |
|---|---|---|---|
| | N | Median | 5-95$^{th}$ percentiles |
| Males (20-49 years) | 241 | 33.2 | 18.3-54.1 |
| Males (≥ 50 years) | 174 | 40.6 | 20.6-76.7 |
| Females (20-49 years) | 166 | 67.8 | 32.4-128 |
| Females (≥ 50 years) | 177 | 62.4 | 27.1-128 |

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

**Specific performance data**

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

| **cobas e** 402 and **cobas e** 801 analyzers | | | | | |
|---|---|---|---|---|---|
| | | **Repeatability** | | **Intermediate precision** | |
| Sample | Mean nmol/L | SD nmol/L | CV % | SD nmol/L | CV % |
| Human serum 1 | 3.03 | 0.0732 | 2.4 | 0.105 | 3.5 |
| Human serum 2 | 10.7 | 0.160 | 1.5 | 0.236 | 2.2 |
| Human serum 3 | 20.9 | 0.351 | 1.7 | 0.495 | 2.4 |
| Human serum 4 | 96.3 | 2.13 | 2.2 | 2.75 | 2.9 |
| Human serum 5 | 171 | 5.23 | 3.1 | 6.44 | 3.8 |
| PC[b)] Universal1 | 53.5 | 1.03 | 1.9 | 1.41 | 2.6 |
| PC Universal2 | 28.0 | 0.544 | 1.9 | 0.681 | 2.4 |

b) PC = PreciControl

**Method comparison**

A comparison of the Elecsys SHBG assay, REF 07258496190 (**cobas e** 801 analyzer; y) with the Elecsys SHBG assay, REF 03052001190 (**cobas e** 601 analyzer; x) gave the following correlations (nmol/L):

Number of samples measured: 133

| Passing/Bablok[19] | Linear regression |
|---|---|
| y = 0.996x + 0.165 | y = 0.996x + 0.0209 |
| τ = 0.977 | r = 0.999 |

The sample concentrations were between 1.14 and 191 nmol/L.

A comparison of the Elecsys SHBG assay, REF 07258496190 (**cobas e** 402 analyzer; y) with the Elecsys SHBG assay, REF 07258496190 (**cobas e** 801analyzer; x) gave the following correlations (nmol/L):

Number of samples measured: 209

| Passing/Bablok[19] | Linear regression |
|---|---|
| y = 1.047x - 0.464 | y = 1.048x - 0.527 |
| τ = 0.985 | r = 0.999 |

The sample concentrations were between 1.15 and 192 nmol/L.

**Analytical specificity**

For the monoclonal antibodies used, the following cross-reactivities were found:

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| Substance | Maximum concentration tested mg/L | Cross-reactivity % |
|---|---|---|
| Transferrin | 20000 | n. d.[c)] |
| Fibrinogen | 50000 | n. d. |
| CBG | 100 | n. d. |
| TBG | 200 | n. d. |
| TG | 1000 | n. d. |
| TSH | 0.0125[d)] | n. d. |
| AFP | 10 | n. d. |
| Testosterone | 0.05 | n. d. |
| E2 | 0.005 | n. d. |
| DHT | 0.05 | n. d. |
| Plasminogen | 3000 | n. d. |
| human IgG | 100000 | n. d. |
| human IgA | 20000 | n. d. |

c) n. d. = not detectable

d) 0.0125 mg/L = 100 µIU/mL

**References**


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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

| | |
|---|---|
| CONTENT | Contents of kit |
| SYSTEM | Analyzers/Instruments on which reagents can be used |
| REAGENT | Reagent |
| CALIBRATOR | Calibrator |
| → | Volume after reconstitution or mixing |
| GTIN | Global Trade Item Number |



CE 0123


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