Sheep sex hormone-binding globulin (SHBG) ELISA Kit

to quantitate Sheep SHBG in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTSH6460EA

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-120-2434
Mobile: +91-98105-21400 | Fax: +91-11-42208444
customerservice@lifetechindia.com

INTENDED USE

This kit is used to determine Canine SHBG in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.03

ASSAY SUMMARY

The kit is used to test the level of Sheep SHBG, based on the principle of double antibody sandwich technology enzyme linked immunosorbent assay (ELISA). The procedure involves addition of standards and samples to the wells that are pre-coated with antibody, then HRP-Conjugate reagent is added to form an immune complex, followed by incubation and washing, removal of unbound enzyme, and then addition of the substrate A and B, which causes the solution to turn blue and finally change into yellow in presence of acid. The color depth or light is positively correlated with the concentration of Sheep SHBG.

CHARACTERISTICS

- This Kit allows for the determination of Sheep SHBG concentrations in Sheep serum, cell culture supernatants and other biological fluids.
- Detection range: 5nmol/L -160nmol/L
- The minimum detectable dose (MDD) of Sheep SHBG is less than 1nmol/L.
- Intra-assay CV(%) is less than 7% and Inter-assay CV(%) is less than 10%.

PRODUCT INFORMATION

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be

returned to cold storage at 4 °C.

Reagents components		96 determinations
1.	Micro Elisa Strip Plate	12*8strips
2.	Standard A	0nmol/L
3.	Standard B	10nmol/L
4.	Standard C	20nmol/L
5.	Standard D	40nmol/L
6.	Standard E	80nmol/L
7.	Standard F	160nmol/L
8.	Sample Diluent	6.0ml
9.	HRP-Conjugate reagent	10.0ml
10.	20X Wash solution	25ml
11.	Chromogen Solution A	6.0ml
12.	Chromogen Solution B	6.0ml
13.	Stop Solution	6.0ml
14.	Closure plate membrane	2
15.	User manual	1
16.	Sealed bags	1

Note: Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to

add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:20.

Sample dilution - If your samples need to be diluted, Deionized or distilled water is used for dilution of samples.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50µl to standard well.

3. Add Sample: Add sample diluent 40 µl to testing sample well. Then add sample 10µl to testing sample well; for Blank well don't add anything.

6. Add 100µl HRP-Conjugate to each well, except blank well.

7. Cover with a adhesive strip. Incubate for 60 minutes at 37 °C.

8. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

9. Add 50 µl chromogen solution A and 50 µl chromogen solution B to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Sheep SHBG concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor (e.g. it is 5 in this case).

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
7. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
8. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
9. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).

THERE MAY BE BATCH TO BATCH VARIATIONS – PLEASE READ CAREFULLY THE MANUAL SUPPLIED ALONG WITH THE KIT

Product of Arsh Biotech Private Limited, Delhi, India