Bovine Progesterone (PROG) ELISA Kit

to determine Bovine PROG in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTOB8100EA

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INTENDED USE

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

MANUAL VERSION 1.02

ASSAY SUMMARY

Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens, and is the major progestogen in the body. Progesterone is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid. This Progesterone ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for progesterone and Horseradish Peroxidase (HRP) conjugated progesterone. The competitive inhibition reaction is launched between with HRP labeled progesterone and unlabeled progesterone with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of progesterone in the sample. The color development is stopped and the intensity of the color is measured.

CHARACTERISTICS

-This Kit allows for the determination of PROG concentrations in serum, cell culture supernates and other biological fluids.

- Detection range: 0.5 ng/ml-30 ng/ml

- The minimum detectable dose (MDD) of PROG is less than 0.5 ng/ml.

- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 9%.

- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians

using two lots of components. The CV (%) < 11%.

- To assess linearity of the assay, samples containing and/or spiked with high concentrations of PROG were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

- This ELISA Kit has high sensitivity and excellent specificity for detection of PROG. No significant cross-reactivity or interference between PROG and analogues was observed.

PRODUCT INFORMATION

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.

-Progesterone microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with antibody.

-Progesterone standard: Progesterone in a buffered protein base with preservatives, liquid.

-HRP conjugated progesterone: liquid.

-Progesterone detect antibody: Antibody specific for progesterone, liquid.

-HRP substrate A: Urea hydrogen peroxide solution.

-HRP substrate B: TMB (Tetramethyl-benzidine) solution.

-Stop solution: 2 mol/L sulfuric acid.

-Wash buffer: PBS with 0.5% Tween-20, 20× liquid.

-Plate covers

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.

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

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 serum/plasma 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. Set a Blank well without any solution.
3. Add 50 µl of Standard or Sample per well. Standard need test in duplicate.
4. Add 50 µl of HRP-conjugate to each well (not to Blank well), then 50 µl detect antibody to each well. Mix well and then incubate for 1 hour at 37 °C.
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (250 µl) using a squirt bottle,

multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, 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.

6. Add 50 µl of Substrate A and 50 µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37 °C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

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 concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the PROG 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.

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. 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.
7. 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.
8. 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.

9. 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).