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SKU LT910011ETKKBA

SHEEP PROGESTERONE ELISA

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.41

INTENDED USE

To detect Sheep PROG in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standard [2 vials]
Standard Diluent [20ml]
Detection Reagent A [1]
Detection Reagent B [120ul]
Reagent Diluent [300ul]
Assay Diluent A and B [12ml each]
30X Wash solution [20ml]
TMB Substrate [9ml]
Stop Solution [6ml]
User manual [1pc]
Zipper bag [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C for unused kit while the others should be at 4°C.

NOT INCLUDED

Distilled Water | Pipettes and Tips | ELISA Reader and 37°C Incubator

SUMMARY

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to analyte has been pre-coated. A competitive inhibition reaction is launched between biotin labeled analyte and unlabeled analyte. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of progesterone in the sample. On addition of the substrate solution, the intensity of color is reverse proportional to the concentration of analyte in the sample

CHARACTERISTICS

Sample Type: Serum, Plasma, Cell Culture Supernatants, Urine, Saliva Biological Fluids, etc.

Detection Range: 1.23ng/ml to 100ng/ml
Minimum Detection Dose: 0.47ng/ml
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%

Cross Reactivity: None observed with analogues.
Validity: 1 year from the date on manufacture
Storage: +2-8°C or -20°C as stated in 'Contents' section

Once Opened unused wells should be put into the sealed bag with a desiccant pack and may be used for up to 1 month.

SAMPLE COLLECTION

Tissue homogenates - 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 and 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. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 5000×g to collect supernatant.

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.

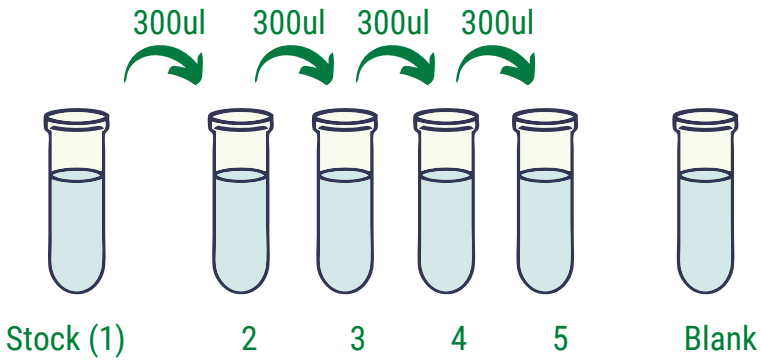
Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

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

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. **Standard** - Reconstitute the Standard with 2.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (do not foam). The concentration of the standard in the stock solution is 100ng/mL and serves as the highest standard. Then prepare 5 more tubes containing 0.6mL Standard Diluent and use the stock solution to produce a triple dilution series as shown below. Mix each tube thoroughly before the next transfer. Thus, set up 5 points of standards: 100, 33.33, 11.11, 3.70, 1.23 ng/mL and keep one more tube with only Standard Diluent to be used as blank (0ng/mL). Prepare standards fresh just before assay.



3. **Detection Reagent A :** Reconstitute the Detection Reagent A with 150ul of Reagent Diluent, keep for 10 minutes at room temperature and shake gently. Dilute to the working concentration with Assay Diluent A (1:100). **Detection Reagent B** - Briefly spin or centrifuge Detection B before use. Dilute it to the working concentration 100-fold with Assay Diluent B.

4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37°C after covering it with the Plate sealer.

4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.

5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.

6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

CALCULATION

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between analyte concentration in the sample and the assay signal intensity.

Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of analyte concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, for instance, curve expert 1.30, is recommended.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at info@arshbiotech.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- 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.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- 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.

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