Creative Diagnostics®

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User's Manual

Pregnant mare serum gonadotrophin (PMSG) ELISA Kit

REF **DEIA2235**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of endogenic pregnant mare serum gonadotrophin (PMSG) concentrations in serum.

Principles of Testing

This assay employs the competitive 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 PMSG and Horseradish Peroxidase (HRP) conjugated PMSG. The competitive inhibition reaction is launched between with HRP labeled PMSG and unlabeled PMSG with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of PMSG in the sample. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Assay plate, 1(96 wells)
2. Standard, 6 × 0.5 ml

   S0: 0 mIU/ml

   S1: 5 mIU/ml

   S2: 17.5 mIU/ml

   S3: 62.5 mIU/ml

   S4: 375 mIU/ml

   S5: 2000 mIU/ml
3. Antibody, 1 × 6 ml
4. HRP-conjugate, 1 × 6 ml
5. Wash Buffer (20 × concentrate), 1 × 15 ml
6. Substrate A, 1 × 7 ml
7. Substrate B, 1 × 7 ml
8. Stop Solution, 1 × 7 ml
9. Adhesive Strip (For 96 wells), 4
10. Instruction manual, 1

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 600 nm - 630 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 mL and 500 mL graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

Storage

Unopened kit: Store at 2 - 8°C. Do not use the kit beyond the expiration date.

Opened kit: May be stored for up to one month at 2 - 8° C.

Specimen Collection And Preparation

1. Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Notes:

1. Creative diagnostics is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Note:

1. Kindly use graduated containers to prepare the reagent.
2. Bring all reagents to room temperature (18-25°C) before use for 30min.
3. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Wash Buffer(1×): If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate (20×) into deionized or distilled water to prepare 300 ml of Wash Buffer (1×).

Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 50µl of Standard or Sample per well. Standard need test in duplicate.
5. Add 50µl of HRP-conjugate to each well (not to Blank well), then 50µl Antibody to each well. Mix well and then incubate for 60 minutes at 37°C.
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) 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.
7. Add 50µl of Substrate A and 50µl 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.
8. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. 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.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 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.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. 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 Substrates.

Calculation

Using the professional soft "Curve Expert" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

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 x-axis against the concentration 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 PMSG 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.

Detection Range

5 mIU/mL-2000 mIU/mL

Sensitivity

5 mIU/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of pregnant mare PMSG. No significant cross-reactivity or interference between pregnant mare PMSG and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection

between pregnant mare PMSG and all the analogues, therefore, cross reaction may still exist.

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.