Equine PMSG / Pregnant Mare Serum Gonadotropin ELISA Kit

Enzyme Immunoassay for the quantification of Horse PMSG / Pregnant Mare Serum Gonadotropin in serum and plasma

Catalog number: ARG81002

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Pregnant Mare Serum Gonadotropin (PMSG) or equine chorionic gonadotropin is secreted by the endometrial cups of the pregnant mares' uterus. The hormone is found in the blood of the pregnant mare between the 40th and 120th days of gestation, reaching a peak at approximately the 60th day. Measurement of PMSG provides a specific test for pregnancy since the hormone is only found in the pregnant mare. After 150 days of gestation, hormone levels are no longer detectable. The PMSG diagnostic test as a means of pregnancy testing is a suitable and convenient method to confirm earlier ultrasound scanning techniques and allows the practitioner to obtain this information quickly and economically in the laboratory.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PMSG has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any PMSG present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-PMSG conjugated antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of PMSG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of PMSG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Standard 0	0.5 ml, ready to use	4°C
Standard 1-5 (25, 100, 200, 400, 800 mIU/ml)	5 X 0.5 ml, ready to use	4°C
HRP-conjugated antibody	11 ml, ready to use	4°C
Assay Buffer	25 ml, ready to use	4°C
40X Wash Buffer	30 ml	4°C
TMB Substrate	14 ml, ready to use	4°C
Stop solution	14 ml, ready to use	4°C

- When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Opened reagents must be stored at 2 °C to 8 °C.
- Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
- Opened kits retain activity for 8 weeks if stored as described above.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm to 630 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly without foaming before use. Avoid repeated freezing and thawing of reagents and specimens.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Be careful to avoid well-to-well contamination. Do not reuse microwells.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at 2-8°C for up to 7 days or store at ≤ -20 °C for up to 1 years. Avoid repeated freeze-thaw cycles. Thawed samples should be inverted several times prior to testing.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately within 4 hours of primary collection or aliquot and store samples at 2-8°C for up to 7 days or store at ≤ -20 °C for up to 1 years. Avoid repeated freeze-thaw cycles. Thawed samples should be inverted several times prior to testing.

NOTE:

- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. E.g. 20 ml of 10X Wash buffer + 780 ml of distilled water to a final volume of 800 ml. The diluted 1X Wash buffer is stable for 1 week at room temperature.
- Sample dilution:

  - If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Assay Buffer and reassayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

  - For pregnant mares a sample dilution of (at least) 1:10 is recommended.

  - Cross reactivity with FSH is eliminated using serum or plasma samples at a high dilution (1:100) with Assay Buffer.

  - Dilution Example:

  a) 1:10 dilution: 10 µL of sample + 90 µL of Assay Buffer (mix thoroughly).

  b) 1:100 dilution: 10 µL of 1:10 diluted samples from a) + 90 µL Assay Buffer (mix thoroughly).

  c) 1:1000 dilution: 10 µL of 1:100 diluted samples from b) + 90 µL Assay Buffer (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **150 µl** of **Assay Buffer** into appropriate wells
3. Add **50 µl** of each **standard and sample** into each appropriate well. Mix the plate thoroughly for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes at RT**.
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.

   Note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µl** of **HRP-conjugated antibody** into each well. Gently tap the side of the plate to mix well.
7. Incubate for **60 minutes at RT**.
8. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X wash buffer** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is

essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.

9. Add **100 µl** of **TMB Substrate** to each well. Incubate for **30 minutes at room temperature in dark.**
10. Add **50 µl** of **Stop solution** into each well.
11. Read the OD with a microplate reader at **450 nm** immediately and read at 620 nm to 630 nm as reference. It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

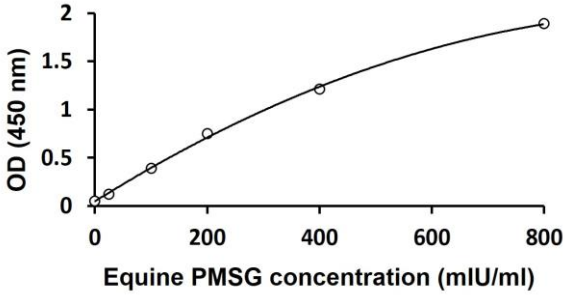
6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 800 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

7. EXPECTED VALUES: The tested serum from Non-pregnant mares (n=27) concentration range was from < 2.0 to 39.2 mIU/ml and the mean

concentration was 12.4 mIU/ml. The tested pregnant mares serum (n=26) concentration range was from 44.2 to > 80000 mIU/ml and the mean concentration was 20232 mIU/ml.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The Limit of Blank (LoB) is 0.89 mIU/mL.

The Limit of Detection (LoD) is 2.02 mIU/mL.

The Limit of Quantification (LoQ) is 5.92 mIU/mL.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.97% and the CV value of inter-assay precision was 8.07%.

Specificity

The following substances were tested for cross-reactivity of the assay:

Hormone tested	Cross-reactivity (%)
Human FSH	9.06
Human LH	0
Human Prolactin	0
Human hCG	0
Human beta-hCG	0.06

Recovery

90.1-114.6%

Linearity

85.1-114.3%

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.063 mg/mL) and Triglyceride (up to 0.47 mg/mL) have no influence on the assay results.