PMSG (Pregnant Mare Serum Gonadotropin) ELISA

For the quantitative determination of PMSG in equine serum and plasma

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 20-PMSEQ-E01
Size: 96 wells
Version: 2.1 – November 20, 2013
Intended Use
The PMSG ELISA is an enzyme immunoassay for the quantitative measurement of Pregnant Mare Serum Gonadotropin (PMSG) in equine serum and plasma

PRINCIPLE of the test
The PMSG ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site of the PMSG molecule. An aliquot of sample containing endogenous PMSG is incubated in the coated well. After washing a second incubation follows with enzyme conjugate, which is an anti-PMSG antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of PMSG in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of PMSG in the sample.

Warnings and Precautions
1. Research Use Only. Not for Use in Diagnostic Procedures.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. 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.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.

16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

17. Avoid contact with *Stop Solution* containing 0.5 M H$_2$SO$_4$. It may cause skin irritation and burns.

18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.

19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.

20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.

Material Safety Data Sheets for this product are available upon request directly from ALPCO.

**Reagents**

**Reagents provided**

1. *Microtiterwells*, 12x8 (break apart) strips, 96 wells; Wells coated with anti-PMSG antibody (monoclonal).

2. *Zero Standard*, 1 vial, 10 mL, ready to use; also used for sample dilution.

3. *Standard (Standard 1-5)*, 5 vials, 1mL, ready to use; Concentrations: for exact values please refer to vial label or QC-Datasheet. Conversion factor: 1 IU ~ 100 ng (1 ng ~ 10 mIU) Contain non-mercury preservative.

4. *Enzyme Conjugate*, 1 vial, 11mL, ready to use, Anti-PMSG antibody conjugated to horseradish peroxidase; Contains non-mercury preservative.

5. *Substrate Solution*, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).

6. *Stop Solution*, 1 vial, 14 mL, ready to use, contains 0.5M H$_2$SO$_4$

Avoid contact with the stop solution. It may cause skin irritations and burns.

**Materials required but not provided**

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction
Storage Conditions
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 six weeks if stored as described above.

Reagent Preparation
Bring all reagents and required number of strips to room temperature prior to use.

Disposal of the Kit
The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged Test Kits
In case of any severe damage to the test kit or components, ALPCO has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation
Serum or plasma can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Storage and Preparation
If samples cannot be assayed immediately, they should be frozen at -20°C (up to two years). Thawed samples should be inverted several times prior to testing.
**Specimen Dilution**
If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Zero Standard 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 LH and FSH is eliminated using serum or plasma samples at a high dilution (1:100) with Zero Standard.

**Example:**
a) dilution 1:10: 10 µL Serum + 90 µL Zero Standard (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Zero Standard (mix thoroughly).

**Assay procedure**

**General Remarks**
- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- The size of the assay run should be limited. Pipetting of all standards, samples and controls should be completed within 6 minutes.
Test Procedure
Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 100 µL of each Standard, control and samples with new disposable tips into appropriate wells.
3. Incubate for 60 minutes at room temperature.
4. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with tape or distilled water (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense 100 µL Enzyme Conjugate into each well.
6. Incubate for 60 minutes at room temperature.
7. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with tape or distilled water (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µL of Substrate Solution to each well.
9. Incubate for 30 minutes at room temperature.
10. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.
11. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.

   It is recommended that the wells be read within 30 minutes after adding the Stop Solution.

Calculation of Results
1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi-logarithmic 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.
5. 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 such. For the calculation of the concentrations the dilution factor has to be taken into account.
NORMAL VALUES
It is strongly recommended that each laboratory should determine its own values.

Quality Control
Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor.
Performance Characteristics

Specificity of Antibodies (Cross Reactivity)
The following equine hormones and hCG were tested for cross reactivity of the assay:

<table>
<thead>
<tr>
<th>Hormone tested</th>
<th>Concentration</th>
<th>Produced color Intensity Equivalent to PMSG IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG α</td>
<td>10 µg/mL</td>
<td>0,185</td>
</tr>
<tr>
<td>(Purity: &gt; 98 % SDS PAGE)</td>
<td>1 µg/mL</td>
<td>0,021</td>
</tr>
<tr>
<td>PMSG β</td>
<td>10 µg/mL</td>
<td>0,440</td>
</tr>
<tr>
<td>(Purity: &gt; 98 % SDS PAGE)</td>
<td>1 µg/mL</td>
<td>0,046</td>
</tr>
<tr>
<td>FSH</td>
<td>50 ng/mL</td>
<td>0,920</td>
</tr>
<tr>
<td>(Immunological potency: &gt; 90 x the NIH Int. Standard FSH-P-1)</td>
<td>5 ng/mL</td>
<td>0,087</td>
</tr>
<tr>
<td>LH</td>
<td>50 ng/mL</td>
<td>0,505</td>
</tr>
<tr>
<td>(Potency: RRA: 9500 IU/mg of the 2d I.S: Serum Gonadotropin)</td>
<td>5 ng/mL</td>
<td>0,049</td>
</tr>
<tr>
<td>hCG</td>
<td>40 IU/mL</td>
<td>0</td>
</tr>
</tbody>
</table>

Reproducibility

INTRA ASSAY
The within assay variability is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean ± SD (mIU/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>79 ± 5,9</td>
<td>7,5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>312 ± 10,0</td>
<td>3,2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>612 ± 50,2</td>
<td>8,2</td>
</tr>
</tbody>
</table>

INTER ASSAY
The between assay variability is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean ± SD (mIU/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>81 ± 6,9</td>
<td>8,5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>308 ± 22,5</td>
<td>7,3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>605 ± 59,3</td>
<td>9,8</td>
</tr>
</tbody>
</table>
Recovery

Samples have been spiked by adding PMSG solutions with known concentrations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogenous PMSG mIU/ml</th>
<th>Added PMSG (mIU/mL)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>79</td>
<td>300</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>93</td>
</tr>
<tr>
<td>B</td>
<td>312</td>
<td>300</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>103</td>
</tr>
</tbody>
</table>

Linearity

Two serum pools were diluted in Zero Standard.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution factor</th>
<th>Measured conc. (mIU/mL)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Undiluted</td>
<td>312</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>148</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>356</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>182</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>43</td>
<td>96</td>
</tr>
</tbody>
</table>

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Cross reactivity with LH and FSH is eliminated using serum or plasma samples at a high dilution (1:100) with Zero Standard. (See 5.2 Specimen Dilution)

High-Dose-Hook Effect

No hook effect was observed in this test