Product information

Useŕs Manual

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

PMSG ELISA

VET

REF **DE1298**

Σ **96**

Demeditec Diagnostics GmbH
Lise-Meitner-Strasse 2
24145 Kiel – Germany
www.demeditec.com

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.

Table of Contents / Inhaltsverzeichnis

1 INTRODUCTION

1.1 Intended Use

The **Demeditec PMSG ELISA** is an enzyme immunoassay for the quantitative measurement of Pregnant Mare Serum Gonadotropin (PMSG) in horse serum.

1.2 Summary and Explanation

Pregnant Mare Serum Gonadotropin (PMSG) or equine chorionic gonadotropin (eCG) is a heterodimer composed of an alpha and beta subunit as for other pituitary gonadotropins, and is post-translationally glycosylated[1]. The primary structure of the eCG β chain is identical to the equine LH β-chain but shows differential glycosylation in the carboxy terminal part[2] resulting in a six fold increase in biological halftime[3]. eCG is secreted by the endometrial cups of the uterus of pregnant mares. eCG can function as luteinizing hormone in the horse, albeit less robustly than eLH[4,14].The hormone is found in the blood of the pregnant mare between day 40 and 120 of gestation, reaching a peak approximately at day 60. eCG is not detectable or present at very low concentrations in the equine serum before day 37 and after day 150 of gestation[5]. A number of factors have been shown to influence the concentration of eCG in mare's blood between 40 and 120 days of gestation. These include maternal body condition[6], exercise, feeding[5], mare size[5], mare parity[7], intrinsic mare variability[3], paternity of the conceptus[8], fetal genotype, fetal gender[9], twin pregnancy, the degree of folding of the endometrium in the gravid uterine horn at the time of invasion of the chorionic girdle[10] and the uterine environment[11]. eCG concentrations are significantly higher in moderately rather than excessively fed mares. Furthermore, eCG concentrations are significantly higher in nonexercised than in exercised mares between days 60 and 90 of gestation[6,13]. Measurement of PMSG can confirm pregnancy from day 41-97 of gestation.

The PMSG ELISA 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.

2 PRINCIPLE OF THE TEST

The Demeditec PMSG ELISA 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 equine sample containing endogenous PMSG is incubated in the coated well together with Assay Buffer. After incubation, unbound components are washed off. Finally, 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 conjugate 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.

3 WARNINGS AND PRECAUTIONS

1. This kit is for laboratory use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use 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 °C to 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 microtiter plate 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_2SO_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 Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-PMSG antibody (monoclonal).
2. **CAL 0 – 5 Standard (Standard 0 - 5)**, 6 vials, 0.5 mL each, ready to use;
   Concentrations: 0 – 25 – 100 – 200 – 400 – 800 mIU/mL
   The standards are calibrated against the following reference material: WHO International Standard Serum Gonadotropin, Equine (NIBSC 62/001)
   Contain non-mercury preservative.
3. **BUF Assay Buffer**, 1 vial, 25 mL, ready to use;
   Contains non-mercury preservative.
4. **ENZ CONJ Enzyme Conjugate**, 1 vial, 11 mL, ready to use;
   Anti-PMSG antibody conjugated with horseradish peroxidase;
   Contains non-mercury preservative.
5. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
6. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use;
   Contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated);
   See “Reagent Preparation“.

Note: Additional *Assay Buffer* for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 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 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 25 °C) prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 1 week at room temperature.

4.5 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 Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, Demeditec 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.

5 SPECIMEN COLLECTION AND PREPARATION

Horse serum 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.

5.1 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.2 Specimen 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 re-assayed 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 samples at high dilution (1:100).

Example:
a) dilution 1:10: 10 µL sample + 90 µL *Assay Buffer* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Assay Buffer* (mix thoroughly)
c) dilution 1:1000: 10 µL dilution b) 1:100 + 90 µL *Assay Buffer* (mix thoroughly)

6 ASSAY PROCEDURE

6.1 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.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **150 µL *Assay Buffer*** into appropriate wells.
3. Dispense **50 µL** of each ***Standard,* control** and **sample** with new disposable tips into appropriate wells.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Rinse the wells **3 times** with **400 µL** diluted *Wash Solution* per well, if a plate washer is used
   - OR -
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted *Wash Solution* per well for manual washing.
   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!
6. Dispense **100 µL *Enzyme Conjugate*** into each well.
7. Incubate for **60 minutes** at room temperature.
8. Rinse the wells **3 times** with **400 µL** diluted *Wash Solution* per well, if a plate washer is used
   - OR -
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted *Wash Solution* per well for manual washing.
   Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of ***Substrate Solution*** to each well.
10. Incubate for **30 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
12. Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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 that. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 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.

Standard		Optical Units (450 nm)
Standard 0	0 mIU/mL	0.06
Standard 1	25 mIU/mL	0.17
Standard 2	100 mIU/mL	0.49
Standard 3	200 mIU/mL	0.87
Standard 4	400 mIU/mL	1.49
Standard 5	800 mIU/mL	2.24

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own values. In a study conducted with apparently non-pregnant and pregnant mares, using the Demeditec PMSG ELISA the following data were observed:

	n	Mean (mIU/mL)	Median (mIU/mL)	2.5th - 97.5th Percentile (mIU/mL)	Range (min. - max.) (mIU/mL)
Non-pregnant mares	27	12.4	11.4	0.1 – 32.7	< 2.0 – 39.2
Pregnant mares	26	20 232	5877	47.6 – 80 000	44.2 – > 80 000

8 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 or Demeditec directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 2.02 – 800 mIU/mL.

9.2 Specificity of Antibodies (Cross-Reactivity)

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

Substance	Cross-reactivity (%)
LH human	0.00
FSH human	9.06
Prolactin human	0.00
β-hCG human	0.06
hCG human	0.00

9.3 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.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability was determined by measuring each sample 10 times per run (n = 10):

Sample	n	Mean (mIU/mL)	CV (%)
1	10	35.54	5.09
2	10	156.15	5.99
3	10	324.36	4.04
4	10	527.96	8.76

9.4.2 Inter Assay

The between assay variability was determine by measuring each sample 10 times per run for 3 days (n = 30):

Sample	n	Mean (mIU/mL)	CV (%)
1	30	33.15	8.82
2	30	154.71	6.43
3	30	320.79	8.30
4	30	572.54	8.71

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 3 different kit lots (n = 18):

Sample	n	Mean (mIU/mL)	CV (%)
1	18	31.59	10.29
2	18	72.05	3.72
3	18	141.56	4.41
4	18	190.42	10.46

9.5 Recovery

Samples have been spiked by adding PMSG solutions with known concentrations.

The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (mIU/mL)		114.25	211.32	381.87	536.06
Average Recovery (%)		108.1	105.4	95.2	99.8
Range of Recovery (%)	from	99.9	99.9	90.1	97.8
	to	114.6	113.0	99.0	101.7

9.6 Linearity

Samples were measured undiluted and in serial dilutions with assay buffer. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (mIU/mL)		30.67	124.77	504.96	30.56
Average Recovery (%)		101.0	97.5	88.8	109.2
Range of Recovery (%)	from	93.2	89.3	85.1	107.2
	to	108.8	109.0	98.4	114.3

10 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.

10.1 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.

10.2 Drug Interferences
Until today no substances (drugs) are known to us, which have an influence to the measurement of PMSG in a sample.

10.3 High-Dose-Hook Effect
Hook effect was not observed in this test up to a concentration of 16 000 mIU/mL of PMSG.

11 LEGAL ASPECTS

11.1 Reliability of Results
The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Demeditec.

11.2 Therapeutic Consequences
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta