Product information

Information about other products is available at: www.demeditec.com

Progesterone camel ELISA

REF **DEV8833**

Σ **96**

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

CONTENTS

1 INTRODUCTION

1.1 INTENDED USE

The Demeditec Progesterone camel ELISA is an enzyme immunoassay for the quantitative measurement of progesterone in serum and EDTA plasma of camel samples. The validation of this assay was performed with dromedary (*Camelus dromedarius*) samples. For manual processing! The usage of laboratory automats is the user's sole responsibility. The kit is intended for single use only.

1.2 DESCRIPTION OF THE ANALYTE

For efficient management of a camel herd, it is essential to diagnose pregnancy as accurately and as soon as possible after mating so that if the camel is not pregnant, it can be re-mated or covered again. There are several methods that can be used to diagnose pregnancy in camels.
As the corpus luteum is essential to maintain the pregnancy in camels, progesterone hormone level is a very useful tool to monitor pregnancy in camels (1,2) and used as a biomarker of early pregnancy detection in female dromedary camel (3).
In the mated dromedary, serum progesterone concentrations increase from day three after ovulation. If the camel is not pregnant, concentrations rapidly return to basal levels by days 10-12, however, if she is pregnant the progesterone concentrations are maintained for the first 90 - 100 days of gestation. According to some studies, progesterone levels then decrease slightly where they remain until day 300. A further slight decrease then occurs over the next 70 - 80 days followed by a rapid drop on the day before, or the day of parturition. Other studies have shown a gradual decrease in progesterone concentration from 5 months of gestation until parturition (1).
However, it must be emphasized that, regardless of the method used, a single pregnancy diagnosis does not guarantee a birth, especially if done at a very early stage (i.e. before 40 - 50 days post mating). This is due in part to errors in diagnosis, but is also due to the high incidence of early embryo loss seen in these species. Further examinations should therefore be carried out at 3-4 months of gestation to ensure the pregnancy is developing normally (1).

2 PRINCIPLE

The test kit is a solid phase competitive enzyme-linked immunosorbent assay (ELISA) in the microtiter plate format with liquid phase incubation for the quantitative measurement of camel progesterone in serum or EDTA plasma samples.
The microtiter plate is coated with a polyclonal anti-progesterone antiserum. Calibrators and samples are pipetted into the antibody-coated microtiter plate, followed by addition of a Biotin-Labeled Progesterone and Incubation Buffer. After an incubation of 1 h, non-reactive components are removed by a washing procedure of the plate. In the next step Enzyme-Labeled Streptavidine is added. Non-reactive components are removed again by a washing step.
A chromogenic substrate, TMB (3,3',5,5'-tetramethyl-benzidine), is added to all wells. During a 30 minutes incubation, the substrate is converted to a colored end product (blue) by the bound enzyme. Enzyme reaction is stopped by dispensing hydrochloric acid as stop solution (change from blue to yellow). The color intensity is indirect proportional to the concentration of camel progesterone present in the sample. The Optical Density (OD) of the color solution is measured with a microtiter plate reader at 450 nm. A calibrator curve is constructed by plotting OD values against concentrations of calibrators, and concentrations of unknown samples are determined using this calibrator curve.

3 WARNINGS AND PRECAUTIONS

1. This kit is intended for laboratory use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing infectious agents.
3. Before starting the assay, read the instruction for use (IFU) completely and carefully. Use the valid version of the IFU provided with the kit. Be sure that everything is understood.
4. The microtiter plate contains snap-off strips. Unused wells must be stored at 2-8 °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 reservoir. Using a reservoir for dispensing 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 microtiter plate wells thoroughly to ensure good test results. Do not reuse wells.
8. Do not let wells dry during assay; add reagents immediately after completing the washing steps.
9. Allow the reagents to reach room temperature (18-25 °C) before starting the test. Temperature will affect the OD readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable gloves when handling samples 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 lead to slightly different results.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec Diagnostics GmbH or on Demeditec homepage (www.demeditec.com).
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4 REAGENTS

4.1 REAGENTS PROVIDED

1. **SORB MT Microtiter plate**, 12 x 8 (break apart) strips with 96 wells, ready to use; wells coated with polyclonal anti-progesterone antiserum.
2. **CAL 0 – 5 Calibrators**, 6 vials, 0.3 ml each, brownish, ready to use; progesterone in a buffered serum matrix.
   The concentrations of the calibrators are 0, 0.25, 0.74, 2.2, 6.7 and 20 ng/ml.
3. **BIOTIN CONJ Biotin-Labeled Progesterone**, 1 vial, 6.0 ml, clear, ready to use; contains biotin-labeled progesterone in a buffered matrix
4. **INC BUF Incubation Buffer**, 1 vial, 6.0 ml, yellow, ready to use.
5. **ENZ CONJ Enzyme-Labeled Streptavidine**, 1 vial, 11 ml, red, ready to use; horseradish peroxidase-labeled streptavidine in a buffered matrix; containing <0.01% CMIT/MIT and <0.02% MIT.
6. **SUB TMB Substrate Solution**, 1 vial, 22 ml, clear, ready to use;
   contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
7. **STOP SOLN Stop Solution**, 1 vial, 7.0 ml, clear, ready to use; contains 2 N hydrochloric acid solution. Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (10x concentrated), clear; see „Reagent preparation“ (4.3).

4.2 MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Vortex mixer
- Microtiter plate mixer operating at 900 rpm
- Manual or automatic equipment for microtiter plate washing
- A microtiter plate reader capable for endpoint measurement at 450 nm
- Timer
- Deionized water
- Absorbent paper
- Semilogarithmic graph paper or software for data reduction

4.3 REAGENT PREPARATION

Wash Solution:
Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for at least 12 weeks at room temperature (18-25 °C). Precipitates may form when stored at 2-8 °C, which should dissolve again by swirling at room temperature (18-25 °C). The wash solution should only be used when the precipitates have completely dissolved.

4.4 STORAGE CONDITIONS

When stored at 2-8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8 °C. After first opening the reagents are stable for 30 days if used and stored properly. Microtiter wells must be stored at 2-8 °C. Take care that the foil bag is sealed tightly.
Protect Substrate Solution from light.

4.5 DISPOSAL OF THE KITS

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.

4.6 DAMAGED TEST KITS

In case of any severe damage of the test kit or components, Demeditec Diagnostics GmbH has to be informed written, 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 SAMPLE COLLECTION AND PREPARATION

For determination of camel progesterone serum and EDTA plasma are the preferred sample matrices. The procedure calls for 10 µl sample per well.

The sample collection devices must be used in accordance with the manufacturer´s instructions. The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood sample from the moment it is collected until it is assayed. Assay the samples promptly or aliquot and store the samples at < -20°C.
Avoid multiple freeze-thaw cycles.

Samples containing sodium azide should not be used in the assay. This can cause false results.
Furthermore do not use hemolytic, icteric, or lipemic samples.

6 ASSAY PROCEDURE

6.1 GENERAL REMARKS

- All reagents and samples must be allowed to come to room temperature (18-25 °C) 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 calibrator, control or sample in order to avoid cross contamination.
- OD 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 equally elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this IFU.
- Duplicate determination of calibrators, controls and samples is recommended in order to identify potential pipetting errors.
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multistepper, respectively, or an automatic microtiter plate washing system. Do not allow wells to dry between incubations. Do not scratch coated wells during washing and aspiration. Wash and fill all reagents with care. While washing, check that all wells are filled precisely with Wash Solution, and that there are no residues in the wells.
- A calibrator curve must be established for every run.
- For internal quality control we suggest to use **Camel Control Set coded DEV88CC.** For more information, please contact Demeditec Diagnostics GmbH.

6.2 ASSAY PROCEDURE

1. Prepare a sufficient number of microtiter plate wells to accommodate **calibrators**, **controls** and **samples** in duplicates.
2. Dispense **10 µl** of each **Calibrator** **CAL** **0** **–** **5** , **control** and **sample** with new disposable tips into appropriate wells of the microtiter plate.
3. Add **50 µl** of **Biotin-Labeled Progesterone** **BIOTIN** **CONJ** to each well.
4. Add **50 µl** of **Incubation Buffer** **INC** **BUF** into each well.
5. Incubate for **1 hour** at room temperature (18-25 °C) on a plate shaker (900 rpm).
6. Discard the content of the wells and wash **4 times** with **300 µl** diluted **1x Wash Solution**. Remove as much wash solution as possible by beating the microtiter plate carefully on absorbent paper.
7. Add **100 µl** of **Enzyme-Labeled Streptavidine** **ENZ** **CONJ** to each well.
8. Incubate for **30 minutes** at room temperature (18-25 °C) on a plate shaker (900 rpm).
9. Discard the content of the wells and wash each well **4 times** with **300 µl** diluted **1x Wash Solution.** Remove as much as possible **Wash Solution** by beating the **microtiter plate** carefully on absorbent paper.
10. Add **200 µl** of **Substrate Solution** **SUB** **TMB** to all wells.
11. Incubate without shaking at room temperature (18-25 °C) for **30 minutes** in the dark.
12. Stop reaction by adding **50 µl** of **Stop Solution** **STOP** **SOLN** to each well. Mix carefully.
13. Determine the OD of each well at **450 nm.** It is recommended to read the wells within 15 minutes.

6.3 CALCULATION OF RESULTS

1. Calculate the average OD values for each set of calibrators, controls and samples.
2. The obtained ODs of the calibrators (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
3. Using the mean OD value for each sample determine the corresponding concentration from the calibrator 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 calculation method. Other data reduction functions may give different results.
5. The concentration of the samples can be read directly from this calibrator curve.

6.3.1 EXAMPLE OF TYPICAL CALIBRATOR CURVE

Following data are intended for illustration only and must not be used to calculate results from another run.

Calibrator		Optical Density
Calibrator 0	(0 ng/ml)	3.192
Calibrator 1	(0.25 ng/ml)	2.848
Calibrator 2	(0.74 ng/ml)	2.239
Calibrator 3	(2.2 ng/ml)	1.482
Calibrator 4	(6.7 ng/ml)	0.779
Calibrator 5	(20 ng/ml)	0.437

7 EXPECTED VALUES

Blood was collected from gestating and non-gestating female dromedaries of a German camel farm and serum and EDTA plasma samples were assayed according to protocol.
The serum-plasma correlation was determined as follows: R = 0.99

Serum		ng/ml						
Population	**n**	**Range**	**Mean**	**Median**	**p2.5**	**p97.5**	**Upper Cut Off**	**Lower Cut Off**
Non-gestating	23	0.14 - 0.64	0.27	0.25	0.16	0.52	0.52	-
Gestating	23	1.22 - 5.06	3.05	2.83	1.63	4.99	-	1.08

EDTA plasma		ng/ml						
Population	**n**	**Range**	**Mean**	**Median**	**p2.5**	**p97.5**	**Upper Cut Off**	**Lower Cut Off**
Non-gestating	16	0.30 - 1.01	0.47	0.43	0.31	0.94	0.94	-
Gestating	8	2.81 - 6.63	3.81	3.12	2.81	6.48	-	2.81

As the gestation weeks were not known for most cases, the range between the upper cutoff of non-gestating females and the lower cutoff of gestating females is considered a transition zone. We recommend to consider resampling at a later stage.
Laboratories should consider the reference range *as guidelines only*. Because of differences which may exist between laboratories and locales with respect to breed, laboratory technique and selection of reference groups, it is important for each laboratory to establish by similar means the appropriateness of adopting the reference range suggested here.

8 QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibrator curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. 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 external Camel Control Set coded DEV88CC are stated in the QC certificate included in 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 analyzing control values and trends. If the results of the assay do not meet 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, microtiter plate reader, 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 Diagnostics GmbH directly.

9 PERFORMANCE CHARACTERISTICS

9.1 ANALYTICAL SENSITIVITY

The analytical sensitivity of the Progesterone camel ELISA was calculated by substracting two standard deviations from the mean of twenty-two (22) replicate analyses of Calibrator 0. The analytical sensitivity of the assay is 0.13 ng/ml.

9.2 ANALYTICAL SPECIFICITY

The following chemically similar substances have been tested for their cross-reactivity to the specific analyte to evaluate the specificity of the assay. The percentage indicates cross-reactivity at 50% displacement compared to progesterone.

Compound	Cross-reactivity at 50% binding
Testosterone	0.14%
Estriol	<0.02%
Corticosterone	<0.02%
11-Deoxycorticosterone	2.07%
17-OH-Progesterone	2.37%
Androstenedione	0.06%
17β-Estradiol	<0.02%
Cortisol	<0.02%
Estrone	<0.02%
Pregnenolone	5.85%
11-Deoxycortisol	0.04%
DHEA	<0.02%
DHEA-S	<0.02%

9.3 REPRODUCIBILITY

9.3.1 INTRA-ASSAY

The intra-assay variation was determined by 20 replicate measurements of three samples within one run using the Demeditec ELISA. The intra-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	0.40	2.68	3.51
SD (ng/ml)	0.03	0.10	0.24
CV (%)	8.1	3.8	6.8
n =	20	20	20

9.3.2 INTER-ASSAY

The inter-assay variation was determined by duplicate measurements of four samples in twelve different runs using the Demeditec ELISA. The inter-assay variability is shown below:

	Sample 1	Sample 2	Sample 3	Sample 4
Mean (ng/ml)	0.44	2.94	0.96	3.88
SD (ng/ml)	0.06	0.22	0.14	0.36
CV (%)	12.9	7.5	14.7	9.3
n =	12	12	12	12

9.4 LINEARITY

Three samples containing different amounts of progesterone were assayed undiluted and diluted. The percentage linearity was calculated by comparing the expected and measured values for progesterone.

		Sample 1	Sample 2	Sample 3
Concentration (ng/ml)		2.63	2.25	3.27
Average Recovery (%)		101	107	108
Range of Recovery (%)	from	95	81	94
	to	107	128	117

9.5 RECOVERY

Recovery was determined by adding increasing amounts of the analyte to three different serum samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the calibrator curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3
Concentration (ng/ml)		3.09	1.78	2.45
Average Recovery (%)		78	90	88
Range of Recovery (%)	from	76	78	83
	to	80	102	90

10 LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the IFU and with adherence to GLP (Good Laboratory Practice). Any improper handling of samples or modification of this test might influence the results.

10.1 INTERFERING SUBSTANCES

- Do not use any hemolytic, icteric or lipemic specimens to avoid any interferences.
- Biotin was tested up to a concentration of 16 ng/ml and no interference with the test was found.
- Samples containing sodium azide should not be used in the assay.
- Non-specific interferences with this immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing.

10.2 DRUG INTERFERENCES

Until today no substances (drugs) are known to us, which have an influence to the measurement of camel progesterone in serum or EDTA plasma. The determination of progesterone can be invalidated if the subject was treated with natural or synthetic steroids. Any medication should be taken into account when assessing the results.

11 LEGAL ASPECTS

11.1 RELIABILITY OF RESULTS

The test must be performed exactly as per the manufacturer's IFU. 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 a sufficient number of controls within the test procedure for validating the accuracy and precision of the test. The test results are only valid if all controls meet the specified ranges and all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Demeditec Diagnostics GmbH.

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 therapeutic consequences should 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.

12 REFERENCES

1. Skidmore L.
   Pregnancy Diagnosis in Camels
   Recent Advances in Camelid Reproduction, Jun 17, 2000
2. Kamoun M. & Jemmali B.
   Serum progesterone level of camel (*Camelus dromedaries*) according to the physiological status
   Journal of New Sciences (2014), Volume 3 (2)
3. Faraz A., Yaqoob M., Tauqir NA, Ishaq HF, Balla AB, Ismail A., Akbar MA, Waheed A and Nabeel MS
   Utilization of hormonal biomarkers for early pregnancy diagnosis in Marecha camel und semi-intensive management system
   Punjab University Journal of Zoology, 37(1): 77-83 (2022)

13 REVISION HISTORY OF INSTRUCTION FOR USE

Version 1 - no former version available

14 SHORT INSTRUCTION

all steps at RT (18-25°C)
all sample sizes given in µl

Steps	CAL 0-5 (0 - 20 ng/ml)	Sample
Pipet **CAL 0-5**	**10**	**-**
Pipet Samples	**-**	**10**
Pipet **BIOTIN|CONJ**	**50**	
Pipet **INC/BUF**	**50**	
Incubate for **1 h** on a shaker (900 rpm)		
Decant Wash **4x** with **WASH|SOLN 1X**	**300** (4 times)	
Pipet **ENZ|CONJ**	**100**	
Incubate for **30 min** on a shaker (900 rpm)		
Decant Wash **4x** with **WASH|SOLN 1X**	**300** (4 times)	
Pipet **SUB|TMB**	**200**	
Incubate without shaking for **30 min** in the **dark**		
Pipet **STOP|SOLN**	**50**	
Read at **λ = 450 nm**		

SYMBOLS USED WITH DEMEDITEC ELISA

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
Σ	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