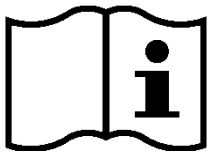

Userś Manual

Progesterone

ELISA

A monoclonal mediated antibody ELISA to detect progesterone in serum or plasma samples of dogs

REF AS1004-ST01

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Test kit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

Determination of the fertile period of the bitch. The visible signs of the fertile period of the bitch (vaginal swelling and excretion) are only rough indications of the ovulation moment. Ovulation is induced by the luteinizing hormone (LH). The day on which the LH-peak reaches its maximum is day 0, the ovulation will occur 2 days after this LH¬ peak. After the ovulation, the egg needs 2-3 days too ripe. The ripe egg will only live for 48-72 hours.
The most fertile period of the bitch is there for 5-6 days after the LH-peak. The rising of the progesterone level correlates with the LH-peak, so the ovulation can be determined accordingly. The progesterone test uses a color reaction.

Other possibilities of the progesterone test:

- detection of luteal cyst (progesterone will stay low)
- detection of ovulation in bitches without heat symptoms
- determination of the most favourable moment for a caesarean operation (progesterone drop below 5 ng/ml about 24 hours before going into labour)
- guarding treatment effects of prostaglandin and progestagen

This progesterone test kit is simple and precise enzyme immunoassay method. By measuring progesterone breeders are able to detect oestrus (luteal function).

2. Principle of the test kit

The Progesterone test kit is based on monoclonal antibodies against a epitope of Progesterone, which are coated to the solid phase. If the sample contains the progesterone hormone, the progesterone will bind to the monoclonal antibody (solid phase).If the sample contains a low level of progesterone the conjugate will bind to the solid phase. If the sample contains a high level of progesterone the conjugate will not bind to the solid phase. When substrate is added color will develop at the samples with low levels of progesterone, if the sample contains a high level no color will develop.

3. Contents

- 12 x 8 Microtiter strips coated with monoclonal anti progesterone
- 1 x Strip holder
- 1 x 8 ml Progesterone buffer (zero standard)
- 1 x 8 ml Anti progesterone HRPO conjugaat (red cap)
- 1 x 1,0 ml Standard containing 20ng/ml progesterone (yellow cap)
- 1 x 20 ml Wash solution (200x concentrated) (black cap), **diluted in de-ionized water before use!**
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop solution (yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

4. Handling and storage of specimens

The kit should be stored at 4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

5. Wash protocol

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down and empty the wells with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

6. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

7. Test protocol Qualitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days. Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water!

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Make a 2-step dilution with a final volume of 75µl of the standard containing 20ng/ml progesterone (yellow cap) in **ELISA buffer** (green cap) **starting undiluted → 1:2 → 1:4 → 1:8** in a round-bottomed microtiter plate (not supplied).

3. Add **75µl of sample** (serum/ plasma) to the remaining wells.
4. Add **75µl of conjugate** to each well. Mix the reagents gently (see **"preparations"**).
5. Incubate for 50 minutes at room temperature.

6. Turn on the analyser (when available).
7. Wash the test strips with running tap water: Fill all wells to the rim. Empty the wells.
8. Repeat 5 times. Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper.
   Take care that none of the wells dry out before the next reagent is added.

9. Add **75µl of substrate A** to each well.
10. Add **75µl of substrate B** to each well.
11. Mix the reagents gently (see **"preparations"**).
12. Incubate for 10 minutes in the dark (e.g. cover the wells with a sheet of paper.
    Read the absorbency values immediately (within 10 min!) at 620 Nm on the analyser or by eye.
    Note: in case of using stop solution read the absorbency at 450Nm on the analyser.

8. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

9. Interpretation of the test results

The analyser will give the results ng/ml, but always double-check the outcome by observing the intensity of colour development.
The progesterone concentration in the samples can be determined by relating them to the standards.
The degree of colour development is proportional to the progesterone concentration.

Colour	Progesterone level	Result
Dark blue	0-2 ng/ml	Basic progesterone level, re-test every 2 days until a blue to a light-blue colour appears.
Blue	4 ng/ml	Mate / inseminate within 5 to 7 days (with frozen semen 3 to 7 days)
Light blue	8-10 ng/ml	The eggs begin to ripen. Mate / inseminate after 2-3 days
No colour	≥12 ng/ml	Mate / inseminate within 24-36 hour

In case of no analyser;
Draw a standard curve by placing the standards (20, 10, 5, 2.5, 0) on the X- as and the O.D. on Y- as on linear/ log paper.
Read the progesterone values from the curve.

10. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso 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.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	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	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

The entire risk as to the performance of these products is assumed by the purchaser. EVL shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products In case of problems or questions contact EVL.

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