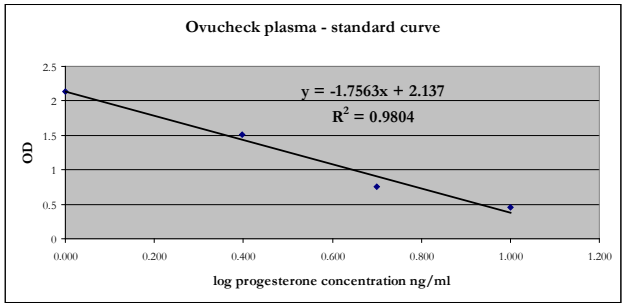
INTERPRETATION

Results

Set spectrophotometer or plate reader to read absorbance at 405 nm. Zero instrument on air. Draw a standard curve by plotting standard absorbance values on the graph paper provided or by using appropriate data analysis procedure (e.g. linear regression using Excel software or 5-parameter logistic regression using for example www.elisaanalysis.com). The progesterone concentration of the controls and samples can be read directly from this standard curve. An Excel spreadsheet is available on Biovet website at : www.biovet.ca/kits. **Please note that the limits of validity of the tests are within the range ≥ 1 to ≤ 10 ng/mL. If you want to measure progesterone concentration > 10 ng/ml you need to use additional standards > 10 ng/mL (please contact Biovet if required).**

Standard Curve Example

P4 standard (ng/ml)	OD (405 nm)
1	2.141
2.5	1.507
5	0.758
10	0.459



Guidelines for the detection of estrus

Species	Progesterone (ng/mL)	Suggested day of sampling for testing
Cow	< 2	19

Bitch: follow-up of blood progesterone concentration to predict ovulation day and optimal breeding day

Progesterone (ng/mL)	Estrus cycle phase	Interpretation
< 3	Proestrus	Retest in 2 days.
3-10	Estrus	Retest in 1 or 2 days.
> 10	Luteal phase	Mate within 48 hours after the progesterone level reaches 10 ng/mL. Vaginal cytology: - If predominance of cornified epithelial cells, mate immediately. - If predominance of intermediate and parabasal cells, it is too late to mate.

Specificity

The interference (cross-reactivity) from steroids other than progesterone is insignificant (less than 1%) except for:

11α-Hydroxy-progesterone	66.0%
5-Pregnan-3β–ol-20-one	16.0%
Deoxy-corticosterone acetate	3.0%
5β-Pregnan-3, 20-dione	4.5%
5α-Pregnan-3, 20-dione	3.3%

Biovet Inc. 4375, Beaudry ave, St-Hyacinthe (Qc) J2S 8W2 Canada
Tél.: 1-888-8BIOVET Fax: (450) 771-2996 **Technical services: support@biovet-inc.com** www.biovet.ca

Measurement of Progesterone in Plasma or Serum
Ovucheck® Plasma
Insert

2016-08-16

The OVUCHECK® PLASMA ELISA kit is an immunoenzymatic test which provides a simple, reliable and precise measurement of progesterone in plasma or serum of **cows** and **bitches in the range of progesterone concentrations ≥ 1 to ≤ 10 ng/mL**.

Each kit contains sufficient reagents for up to 92 tests plus four standards.

OVUCHECK® PLASMA is used for oestrus detection and assessment of pregnancy status and luteal function.

PRINCIPLE OF THE TEST

The OVUCHECK® PLASMA test is based on the competitive binding of unlabelled progesterone present in the standard or sample, and a fixed quantity of progesterone labelled with the enzyme alkaline phosphatase (AP) (conjugate), to binding sites on a limited amount of specific progesterone antibodies.

The wells are pre-coated with specific progesterone antibodies, providing a solid phase for the capture of the progesterone present in the samples, the standards or the conjugate. After incubation, all components other than those bound to the plate wells are washed away.

The amount of bound AP-labelled progesterone remaining in the wells is inversely proportional to the concentration of the unlabelled progesterone present in the sample. The bound labelled progesterone is then measured by making the AP react with its substrate during a second incubation.

The colour produced is measured spectrophotometrically and the concentration of progesterone in the sample is determined from a standard curve.

PRECAUTIONS

- Store the kit at 2-7°C. DO NOT FREEZE.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The standards and the conjugate contain a preservative. When emptying the wells' contents into a sink, thoroughly flush away with an excess volume of tap water.
- For *in vitro* veterinary diagnostic use only. The components or their residues must not be allowed to come into contact with livestock.
- Do not pipet by mouth.
- If eyes or skin are splashed, wash thoroughly with tap water.
- The material used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Dispose of the substrate and the stop solution according to local regulations for chemicals.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with progesterone antibodies	1
• Ready-to-use progesterone standards ** (Each of: 1.0; 2.5; 5.0; 10.0 ng/mL)	1.0 mL
• Ready-to-use conjugate-(Prog.-AP)	26 mL
• Ready-to-use substrate ***	25 mL
• Ready-to-use stop solution	20 mL
• Graph paper	1

****It is important to store the standard tubes in UPRIGHT position in order to prevent contact of the standards with the inner of the cap**
***** Substrate containing black insoluble stabilizing pellets.**

Materials Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- ELISA 96-well microplate reader equipped with 405 nm filter

EXECUTION

A. Sample Collection

Plasma:

- Blood samples should be collected into heparinised collection tubes (do not use EDTA tubes).
- Centrifuge the blood sample within 30 minutes of collection and draw off the plasma into a clean container. The plasma sample can be kept in the refrigerator for up to one week before testing or in the freezer for many months.
- If centrifugation is not immediately available, keep the blood sample as cool as possible and centrifuge within 24 hours of collection. Progesterone levels fall by as much as 30% under these conditions.

Serum:

- Blood samples should be collected into a non-heparinised tube (do not use serum separator tubes). Once the clot has formed, centrifuge the tube within 30 minutes and draw off the serum into a clean container.
- If a centrifuge is not available, the serum should be drawn off as soon as the clot has settled. Serum samples can be kept in the refrigerator for up to one week before testing, or in the freezer for many months.

B. Test Procedures

1. Bring all components to room temperature (22 ± 3°C) before use (approximately 30 minutes).
2. Homogenize samples and reagents just before use except substrate which is important to not homogenized (cf #11)
3. Free the microtitration plate from the protective packaging. Take the quantity of microwells needed for the test (4 wells for the controls and 1 well per sample to be tested). Wells can be separated from one another. Return unused microwells strips to storage at 2-7°C.
4. To prevent any mistake, it is recommended to identify the wells used by making a schematic representation.
5. It is important to distribute samples and reagents in the wells in the minimum amount of time required (less than 10 minutes) to ensure a similar reaction time in all wells. If there are too many samples test them in smaller batches.
6. Add 10 µL of each four standards to be used to the appropriate wells.
7. Add 10 µL of each sample to be tested to the appropriate wells.
8. Immediately add 200 µL of conjugate to every used well. Take care not to contaminate the micropipette tips with the samples. Gently shake the wells to homogenize reagents.
9. Incubate for 30 minutes at room temperature away from light.
10. Empty wells and gently wash them with a pipette or a wash bottle using purified water at room temperature. Repeat twice more. Tap dry on absorbent paper. Do not let the wells dry completely.
11. Add 200 µL of substrate to all wells. Take care not pipetting black pellets, pipet supernatant only
12. Incubate for 30 minutes at room temperature away from light.
13. Add 100 µL stop solution to all wells.
14. Read the results at 405 nm. The reading should be done no later than 15 minutes after the addition of the stop solution. See the « RESULTS » section.
15. At the end of testing, return components to storage at 2-7°C.