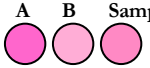
INTERPRETATION

Progesterone level in serum or plasma varies depending on the physiologic stage.

In non cyclic animals (e.g. prepubescent gilts, sows in anoestrus) and just around oestrus (heat) progesterone level is very low (0 to 2 ng/mL).

Progesterone level quickly increases (> 2.5 ng/mL) within 24 – 48 hours after ovulation and remains high (> 5 ng/mL) for about 15 to 18 days.

Progesterone level rapidly falls before the onset of a new cycle. This fall does not occur if the animal is pregnant.

Test Result		Interpretation
A B Sample 	Sample has same colour or is darker than standard A (2.5 ng/mL)	Low level of progesterone (< 2.5 ng/mL). Absence of active corpora lutea. E.g.: animal is not cycling, is in oestrus (heat) or is close to oestrus.
A B Sample 	Sample tested is paler than standard A (2.5 ng/mL) but darker than standard B (5.0 ng/mL)	Medium level of progesterone (between 2.5 and 5.0 ng/mL). Presence of active corpora lutea. E.g.: animal has recently ovulated or is at the end of the luteal phase (before the onset of a new cycle).
A B Sample 	Sample has same colour or is paler than standard B (5.0 ng/mL)	High level of progesterone (> 5 ng/mL). Presence of active corpora lutea. E.g.: animal is in the luteal phase, is pregnant or has luteal cysts.

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 Swine Plasma or Serum
Ovucheck® Premate Porcine
Insert

2017-01-18

OVUCHECK® PREMATE PORCINE is an immunoenzymatic (ELISA) kit which provides a simple and reliable measurement of progesterone in plasma or serum of female swine (gilts, sows).

OVUCHECK® PREMATE PORCINE is intended for assessment of ovarian function and provides a reliable aid to sow reproduction management.

Each kit contains sufficient reagents for up to 28 tests.

PRINCIPLE OF THE TEST

The OVUCHECK® PREMATE PORCINE 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 on 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 obtained with the sample is compared to that produced by two standards of 2.5 and 5.0 ng of progesterone/mL.

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 well 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 pipette 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 according to local regulations for chemicals.

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MATERIAL

Components	Quantity
• Strips of 8 wells coated with anti-progesterone antibodies	4
• Ready to use progesterone standard 2.5 ng/mL (A) *	1 mL
• Ready to use progesterone standard 5.0 ng/mL (B) *	1 mL
• Ready to use conjugate (C)	8.5 mL
• Substrate buffer (D)	8.5 mL
• Substrate tablet	1
• Plastic pipettes	35
• Dropper stopper	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

Materials Required but not Provided:

- Tap water
- Absorbent paper

EXECUTION

A. Substrate Preparation

Add the substrate tablet into the substrate buffer bottle (D). Screw the dropper stopper on the substrate buffer bottle (D). Shake until complete dissolution (15 to 30 minutes may be necessary). Keep the substrate away from light.

If all the substrate is not required immediately, it can be dispensed into clean plastic containers and stored at 2-7°C for 3 months or frozen (-20°C) until expiration of the kit.

B. Sample Collection

Serum:

- Blood samples should be collected into a non-heparinised tube (do not use serum separator tubes). One mL of blood is enough. Once the clot has formed (about 1 hour at room temperature or one night at 2-7^{0}C), centrifuge the tube 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 (2-7^{0}C) for up to one week before testing, or in the freezer (-20°C) for many months.

Plasma:

- Blood samples should be collected into heparinised collection tubes (green stopper). Do not use EDTA tubes. One mL of blood is enough.
- Centrifuge the blood sample within 30 minutes of collection and draw off the plasma into a clean container. Plasma sample will remain usable for up to for up to 24-48 hours in a refrigerator (2-7^{0}C) or many months if kept frozen (-20^{0}C).
- If centrifugation is not immediately available, keep the blood sample in a refrigerator (2-7^{0}C) and centrifuge within 24 hours of collection.

C. Test Procedures

1. All samples and kit components have to be brought to room temperature (30 minutes to two hours may be necessary).
2. Take out a strip of wells from the plastic bag.
3. Select the required number of wells by breaking the plastic between the wells. Use 2 wells for the standards and one well per sample to be tested.
4. **It is not recommended to test more than 14 samples at the same time.**
5. Put the unused wells back in the plastic bag and return them to storage.
6. For identification purposes mark the top of the first well you will use. To prevent any mistake, it is recommended to identify the wells used by making a schematic representation.
7. Shake samples and standards just before use.
8. Using a pipette and keeping it vertical, add one drop of standard A to the first well, one drop of standard B to the second well and one drop of each sample to be tested to the appropriate wells. Always use a new pipette for each sample or standard. Wells must contain a standard or a sample, not both.
9. Keeping bottle C vertical add 4 drops of reagent C (conjugate) to each well.
10. Put the wells into the dark (e.g. place into an opaque box or wrap up with an aluminium foil) and let stand for 15 minutes at room temperature.
11. Empty the contents of the wells into the sink and gently rinse the wells three times using tap water. Dry by taping onto absorbent paper.
12. Keeping bottle D vertical add 4 drops of substrate (see section A) to each well.
13. Agitate the wells gently to mix the contents.
14. Put the wells into the dark (see above #10) and let stand for 15 minutes at room temperature.
15. Place the wells on a sheet of white paper and observe them from above.
16. Verify that a pink colour did appear in the standard wells and that standard A (2.5 ng/mL) is darker than standard B (5.0 ng/mL).
17. If so, tap the wells to mix contents evenly and compare the colour of each sample well with those of the two standard wells.