

P4^{FPA}

PROGESTERONE TEST FOR BOVINE MILK

The P4^{FPA} is a semi-quantitative test that uses competitive Fluorescence Polarization technology. It determines the level of progesterone in milk samples from cattle.

Progesterone is a steroid hormone produced by the corpus luteum, a temporary endocrine gland on the mammalian ovary with specific up- or down-regulation during the estrus cycle. Progesterone is a non-specific hormone that can detect pregnancy. It can also signal estrus timing with a low concentration occurring 2-3 days before oncoming estrus.

Note

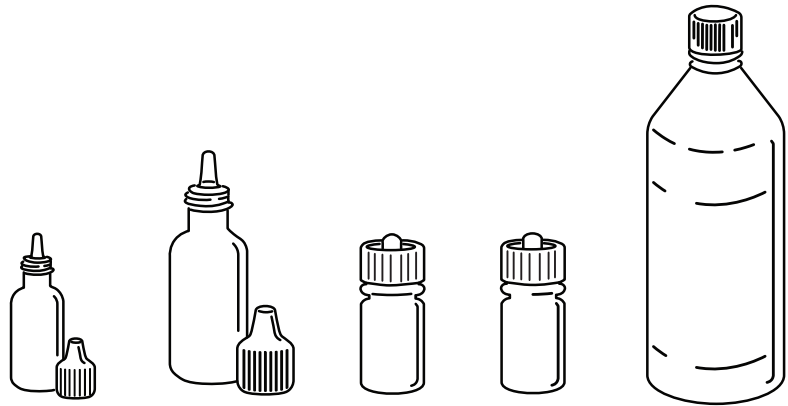
Before using this information and the product it supports, read the Warning and Sample Requirements. Following these instructions is critical for the successful use of this product.

First Edition (January, 2020)

© Copyright ELLIE LLC 2020

All rights reserved.

Kit Contents



PC
Positive
Control

CM
ClearMilk™
Buffer

T
Tracer
(Conjugate)

D
Detector

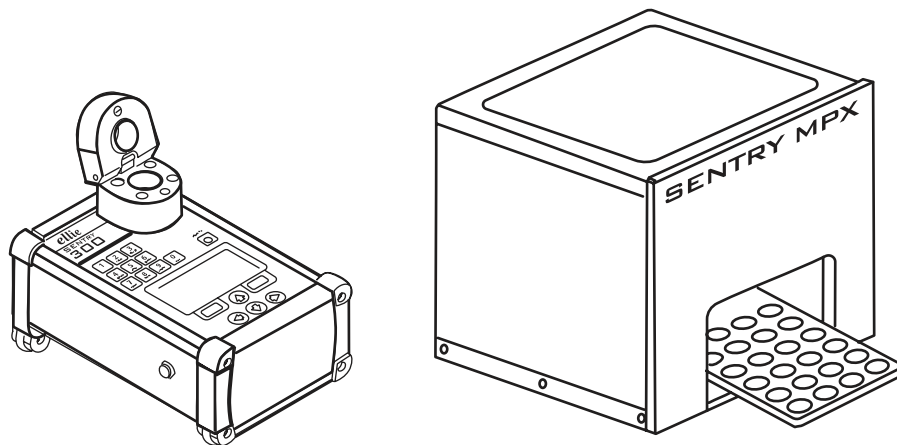
SD
Sample
Diluent

250 test kit	4 ml	60 ml	2.5 ml	2.5 ml	250 ml
1000 test kit	10 ml	2 × 60 ml	10 ml	10 ml	1000 ml

Materials Required but not Provided

Instruments

To read the reaction, you will need an FP instrument of which we manufacture two types. The Sentry 200 or 300 series of instruments read one tube at a time, and the Sentry MPX reads microplates, which are plastic trays containing 96 or 24 test wells. Instruments are available for loan or purchase, depending on the testing volume. Sentry 200/300 instruments are portable, battery-powered, and can be used in the field. They are suitable for testing up to 100 samples per day. The Sentry MPX microplate instrument is stationary and can process up to 1000 samples per day. Ellie does not recommend using other FP instruments and does not provide a warranty for the P4F-PA assay if other instruments are used.

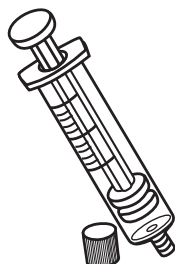


Other materials

- 1.5 ml microcentrifuge tubes when using the syringe/filter method for sample treatment
- 2 ml microcentrifuge tubes when using the centrifugation method for sample treatment
- 20 ml Luer lock syringes
- Syringe stopper
- 0.2- to 0.7-micron 25 mm syringe filter made of glass fiber, nylon, or PES
- 10 x 75 or 12 x 75 mm glass test tubes (cat # T1075 and T1275) or 24-well black microtiter plates (cat # PLATES24), depending on the instrument choice
- Pipettes and pipette tips
- Absolute ethanol
- Microcentrifuge
- Vortex, if available
- Manual repeating pipette (e.g. Eppendorf Repeater M4, cat # 4982000322)
- Tips for the repeating pipettor to deliver 10 μ l of liquid (e.g. Eppendorf Combitips 0.5 ml, cat # 0030089421)



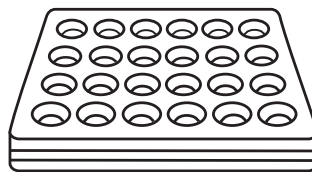
1.5 or 2 ml
microcentrifuge
tubes



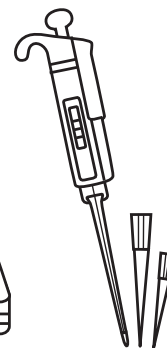
20 ml luer-lock
syringes
and stoppers



10 x 75 mm
or 12 x 75 mm
borosilicate glass
test tubes



24-well black
microplates



1 ml and 10 μ l
micropipettes
and tips



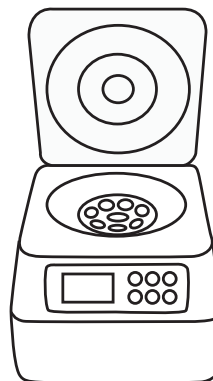
syringe
filters



absolute
ethanol



repeating pipette
(optional)



microcentrifuge
~10-15000 RPM
(optional)



vortex mixer
(optional)

Storage & Stability

Store at 2-8°C. The kit is transported in a cooled box at a temperature between 0 and 15°C.

Warnings

Positive Control can easily contaminate pipettes or other reagents. Handle with care and avoid contact with liquid.

Polarization readings are affected by temperature. All reagents used during the test should be at the same temperature as the samples being tested. Bring all reagents and samples to room temperature.

Use good quality, calibrated pipettes. Avoid practices that may contaminate reagents.

All materials in this kit should be treated according to the MSDS. Avoid ingestion, eye contact, skin contact, and other potentially detrimental exposure. All components contain less than 0.1% sodium azide.

Do not use expired or contaminated components or components from other kits. Do not mix components from different manufactured lots.

Instruments used to generate test results must be obtained from or approved by Ellie. Warranty or performance is not guaranteed otherwise.

Specimen Requirements

Samples should be taken before the morning milking. Take milk sample from the healthy quarter of the udder by throwing away first 10-15 jets and then start to collect in a clean container. Collect minimum 50 ml of milk.

Samples taken at the end of milking process, right after the milking process or a few hours after the milking process are not suitable for P4^{FPA} assay.

Milk samples from the quarter of the udder with signs of mastitis, are not suitable. The most obvious symptoms of clinical mastitis in the udder are swelling, heat, hardness, redness or pain. Milk takes on a watery appearance and flakes, clots or pus are often present.

Milk samples should be transported in a cooled box at a temperature between 0 and 15°C with or without added preservative (e.g. potassium dichromate). For short time storage up to 3 days keep the milk samples at 2-8°C. For long time storage keep the milk samples at -20°C

Sample Preparation - Syringe Filter Method

Prepare syringes and vials:

1. Prepare a 20 ml Luer lock syringe for each sample. Place a stopper on each syringe to prevent spillage.
2. Prepare a vial (1.5 ml microcentrifuge tube or similar) for each sample. Place the vial(s) into a tube rack for easier processing.
3. Label the syringes and vials with the corresponding sample IDs.

Treat samples

1

Mix samples well before processing!



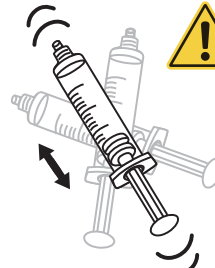
2

Add 3 ml of each sample into a corresponding syringe.



3

Add 1.5 ml of absolute ethanol into each syringe and mix vigorously for ~15 seconds!



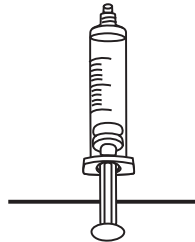
4

Add 3 drops of ClearMilk™ Buffer and mix gently before incubation step.



5

Leave syringes 5-15 min in an upright position for milk to clot.



6

Remove stopper and mount a 0.2 to 0.7 micron 25 mm syringe filter.



7

Push milk through the syringe into a corresponding vial, obtaining clear milk serum for FPA testing.



Sample Preparation - Centrifugation Method

1. Prepare enough microcentrifuge tubes for each sample;
2. Label microcentrifuge tubes with corresponding sample IDs;

Treat samples

1

Mix samples well before processing!



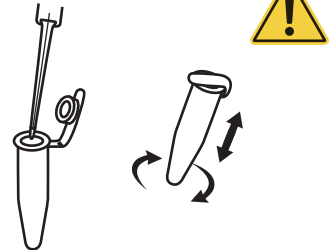
2

Add 1 ml of each sample into a corresponding micro tube.



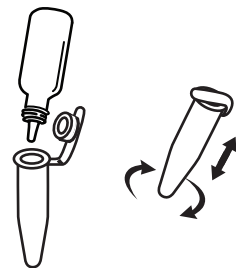
3

Add 500 μ l of absolute ethanol into each tube with samples and mix vigorously for ~15 sec!



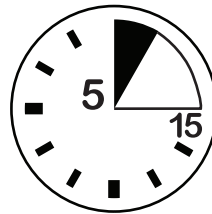
4

Add one drop of ClearMilk™ Buffer and gently mix.



5

Incubate for 5-15 minutes



6

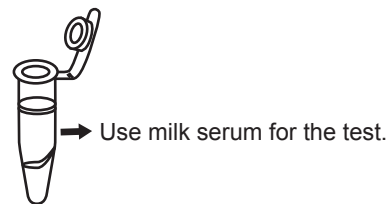
Centrifuge at 10000 g (13-15K rpm) on a typical microcentrifuge for one minute.



7

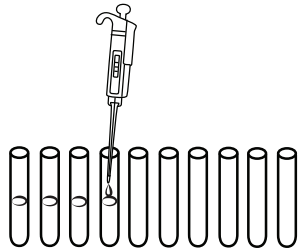
The milk sample will be separated into three layers:

- Lipid layer on the top
- Clear milk serum in the middle
- White casein at the bottom.



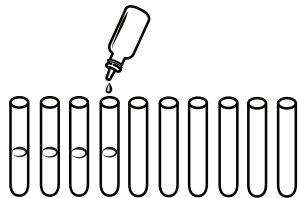
Testing Protocol

The following example is a test protocol for 10 tubes, the first four of which are controls. The first initial run must include controls, but controls are not needed after the first run. However, controls should be run every 30 minutes (due to changes in reagent temperatures) or when you suspect the conditions have changed.



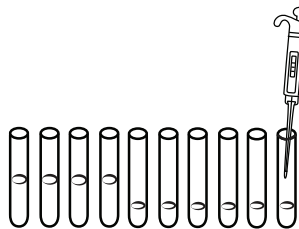
1

Pipette 1 ml of Sample Diluent in four test tubes.



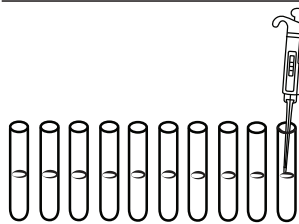
2

Add one drop of the PC into the fourth tube. First three tubes will serve as Negative Control.



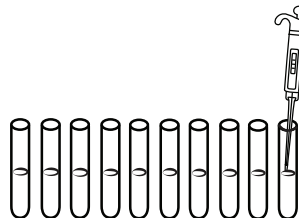
3

Pipette 300 μ l of samples into remaining tubes. Make sure that the outside of pipette tip is clear from fats, if not clean it using clean paper towel and then pipette the sample.



4

Add 700 μ l of Sample Diluent into each tube containing samples. Do not pipette into tubes containing controls. Mix well before next step.

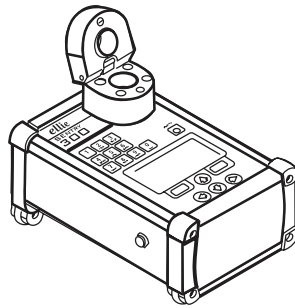


5

Add 10 μ l of Detector into all wells containing controls and samples. Mix well.

The test protocol described above uses an instrument that reads individual tubes. The same reagent volumes are added to the 24-well microplate for processing with a plate reader. When using 24-well plates, include a set of controls on each plate. The well that is used for the Positive Control cannot be reused to test samples or the Negative Control. From this point forward in the protocol, the microplate reader can process samples without human intervention.

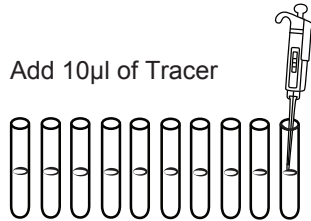
Continue test protocol:



6

Obtain blank readings of controls and samples.

Add 10µl of Tracer



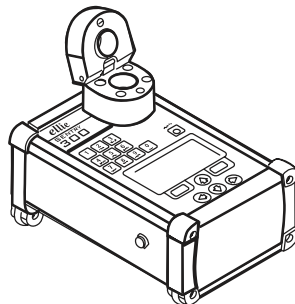
7

Add 10 µl of Tracer into all wells. Mix well all tubes.



8

Incubate for five minutes at room temperature.



9

Obtain final readings of all tubes.

Test Validation

The Negative Control must read between 270 and 300 mP;

The Positive Control must read between 20 and 70 mP;

If either control is outside of the above ranges, the test is considered invalid. Please contact technical support at support@ellielab.com or (800) 556-6953.

Results & Interpretation

Calculate ΔmP values for each sample by subtracting sample mP from mean Negative Control:

$$\Delta mP = (\text{Avg Negative Control mP} - \text{sample mP})$$

The test results are interpreted according to the following table:

ΔmP	0-35	35-45	>45
Progesterone Level	Low	Medium	High

A **Low progesterone level** can indicate different physiological and pathological conditions:

- The cow is in visible or silent estrus/heat
- Ovulation will occur soon
- The cow is not cycling
- The cow is not pregnant

Animals that show a **medium progesterone level** should be retested one or two days later.

A **high progesterone level** can indicate different conditions:

- Possible pregnancy
- The cow is in the proestrus or metestrus phase
- Luteal phase
- Different pathological conditions like persistent corpus luteum, pyometra, fetal mummification, etc.

Notes