# Abraxis Progesterone (bovine) ELISA Kit

Enzyme immunoassay for the quantitative determination of progesterone in bovine milk/serum/plasma samples

REF PN5081M

96 Tests



For Research Use Only.

Not for use in diagnostic procedures



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Abraxis Screen Progesterone ELISA milk/serum/plasm	ia (bovine)	

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The AbraxisScreen Progesterone ELISA is a competitive immunoassay for the determination of progesterone (P4) directly from bovine raw milk or serum/plasma samples. Serum, plasma and milk samples can be analyzed in parallel in the same run.

The AbraxisScreen Progesterone ELISA can be run following two different protocols:

**Quantitative protocol:** up to 42 samples run in duplicates using 6 standards.

**Semiquantitative protocol:** up to 94 samples in single using 2 controls.

# Principle of the Test

The test is based on the specific recognition of progesterone by specific antibodies immobilized on the surface of the microtiter plate. The progesterone present in the standard solution or in the sample competes with a constant amount of a progesterone-enzyme-conjugate for the binding sites of the immobilized antibodies. If the progesterone-enzyme-conjugate reveals a relative higher concentration compared to the free analyte it will predominantly bind to the antibody and vice versa. As a result higher signals will correspond to lower concentrations of analyte.

#### Introduction

The determination of the progesterone level in dairy cattle is a valuable tool for efficient herd management. During the bovine heat cycle the level of progesterone (P4) changes significantly (Figure 1). On day 0 of the oestrus very low progesterone levels can be detected due to an inactive corpus luteum. The corpus luteum develops from an ovarian follicle during the luteal phase leading to production of progesterone and therefore increasing progesterone levels. These high levels even increase further in case of pregnancy after day 20 or drop again indicating the beginning of a new cycle. Consequently the determination of the progesterone level in milk can help in maintaining an accurate time management of artificial insemination (AI) of cows.

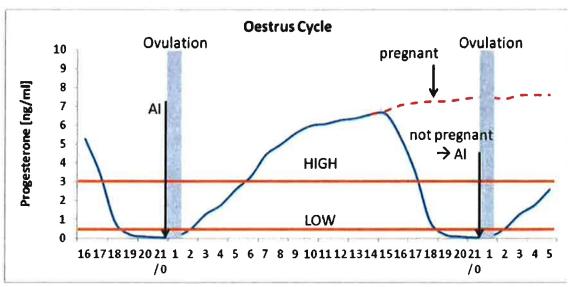


Figure 1: Progesterone levels during the oestrus cycle of a cow. Al should be performed when progesterone levels are low.

# Applications

Application	Description	P4 Level	Interpretation
Oestrus confirmation	Successful artificial insemination (AI) can only be performed during oestrus which is characterized by an absent corpus luteum and low progesterone levels	< LOW	cow in oestrus
Cycle detection	In case of an unknown cycle of the a After 20-24 days the cycle of the cov		
Identification of silent heat or false heat	Animals not showing any heat signs progesterone levels during a fertile for AI.  Animals showing heat signs while be cannot be used for successful AI. Re progesterone levels (< LOW) indicat	oestrus (< L0 earing an act test in 4 day	DW) and can then be used tive corpus luteus (> LOW) intervals until low
Identification of open cows	In order to not lose a cycle for AI a sample on day 20 after AI can be analyzed for progesterone.	< LOW	100% not pregnant. new oestrus can be used for Al.
		> LOW	> 80% pregnant
Confirm or exclude ovarian cysts / monitoring of cyst treatments	Collect samples on day 0, day 7 and day 14. In a normal fertility cycle the progesterone level increases rapidly and then plateaus. If the progesterone level	< LOW	Follicular Cyst: Prolonged low progesterone levels can be an indication for follicular cysts.
	within this period does not vary, the cow should be examined for	> LOW	6 days after treatment the restart of the cycle

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	ovarian cysts.	+	can be monitored by elevated progesterone levels.
		> LOW	Luteal Cyst: Prolonged high progesterone levels can be an indication for luteal cysts.
		< LOW —	2-4 days after successful treatment progesterone levels are low.
Monitoring of cycle synchronization protocols	Successful treatment with prostaglandin can only be performed during an active corpus luteum and will lead to low progesterone levels (< LOW) 4 days after application.	> LOW > HIGH +	Active corpus luteum. Prostaglandin treatment can be performed
Identification of heat detection problems	In case of a low herd fertility the quality of heat recognition should be analyzed by measuring 20 cows immediately after AI (d0) and 4 days after.  If more than one animal is missing the expectations on the given days then the heat detection error is	day 0 < LOW  day 4 > LOW	Al in time
	too high (> 5%). Other reasons for low herd fertility can be due to e.g. stress, feeding.	day 0 + 4 > LOW	Al too late
	2	day 4 < LOW —	Al too early

# Material

# **Material supplied**

- Microtiter plate with plate lid: 12x8 (break apart) strips wells coated with a monoclonal anti-progesterone antibody
- Standard (1-5): 6 vials, 1 mL
   Concentrations 0, 0.23, 0.47, 0.94, 3.75, 20 ng/mL
- Controls: 2 vials, 1 mL
   LOW (0.47 ng/mL); HIGH (3.75 ng/mL)

4. Enzyme Conjugate: 1 vial, 11 mL

Progesterone conjugated to horseradish peroxidase

5. Substrate Solution: 1 vial, 11 mL

6. Stop Solution: 1 vial, 6 mL

9.9 % H<sub>3</sub>PO<sub>4</sub>. Avoid contact with skin and eyes!

7. Wash Solution (15x concentrate): 1 vial, 33 mL

Dilute before use. Content sufficient for preparation of 500 mL 1x Wash Solution.

8. Instruction Booklet

## Other essential materials needed but not supplied

- 1. Deionized or distilled water
- 2. Precision Micropipettes for volumes of 20-200  $\mu$ l and 100-1000  $\mu$ l
- 3. Beakers for diluting the buffers
- 4. A microtiter plate washer (alternatively washing with multi-channel pipette)
- 5. A microtiter plate reader capable of reading 450 nm wavelength (ideally with a reference filter at wavelength 620 nm)

## Preparation of reagents

- Bring all reagents to room temperature before use
- Place all reagents at 2-8 °C immediately after use

#### Wash Solution (15x concentrate):

Dilute the content of the Wash Solution concentrate (33 mL) with 467 mL deionized water to a final volume of 500 mL.

The diluted Wash Solution is stable for 2 weeks a room temperature.

# Warning and Precautions

- Avoid contact with the Stop Solution. It may cause skin irritation and burns.
- Do not use reagents after expiration date.
- Do not mix reagents from different kits.
- Store reagents under refrigeration (2-8 °C). Do not freeze!
- Dispose of kit components in accordance with applicable regulations after use.

# Sample Collection

#### Milk

Milk samples from whole milk or foremilk should be used for testing. Samples are stable for at least 24 h at 2-8°C. For longer storage periods the samples should be frozen at -20°C.

Immediately before analysis, samples should be shaken 2-3 times to generate a homogeneous emulsion.

# Serum/Plasma

Do not use haemolytic, icteric or lipaemic samples.

For plasma: Collect blood samples in heparin collection tubes (EDTA could interfere with the

ELISA) and centrifuge within 30 min after collection. Draw off supernatant in a fresh

vial.

**For serum:** Collect blood samples in serum collection tubes. Allow to clot and centrifuge sample.

Draw off supernatant in a fresh vial.

Samples are stable for at least 24 h at 2-8°C. For longer storage periods the samples should be frozen at -20°C.

#### **Test Procedure**

The AbraxisScreen Progesterone ELISA can be run in two different protocols:

A <u>quantitative protocol</u> for up to 42 samples run in duplicates.

A <u>semiquantitative protocol</u> for up to 94 samples in single.

#### **General Remarks**

- Bring all reagents to room temperature before use.
- All reagents and samples should be mixed before use.
- Use a new disposal pipette tip for every standard, control and sample to avoid contamination.
- Pipetting should be completed without interruption.

# Assay procedure

For the <u>quantitative protocol</u> all standards, controls and samples should be applied in duplicates. For the <u>semiquantitative protocol</u> only controls LOW and HIGH need to be applied and all controls and samples are run in single wells only (standard solutions are not needed there).

- 1. Remove the strips not required in the test from the holder, and place them back in the sealing bag for future use.
- 2. Pipet **25 µ**I of **Standards, Controls** and **Samples** in duplicates on the microplate wells (for the semiquantitative protocol: only pipet controls LOW, HIGH and the samples in single wells)
- 3. Add 100 µl of the Enzyme Conjugate solution to each well
- 4. Mix wells gently
- 5. Cover wells with the provided plate lid
- 6. Incubate for 20 min
- 7. Wash wells 3 times with 300 µl of diluted Washing Solution
- 8. Remove the remaining liquid by patting the plate on a stock of paper
- 9. Add 100 μl of Substrate Solution to each well
- 10. Incubate for 10 min in the dark
- 11. Stop the color reaction by adding 50 μl of Stop Solution
- 12. Measure at 450 nm (with a reference filter at 620 nm) within 30 min

## Results

## Semiquantitative protocol

Instead of stopping the reaction with stop solution and measuring the OD values using a photometer, the blue shades of the samples can be visually compared with the two controls LOW (0.4 ng/ml) and HIGH (3.75 ng/ml) and can be classified in their progesterone content:

OD values	P4 level	ng/ml	classification
> LOW (darker blue)	low	< 0.47	-
<b>ODs between LOW and HIGH</b>	medium	0.47-3.75	+
ODs < HIGH (lighter blue)	high	> 3.75	++

## **Quantitative Protocol**

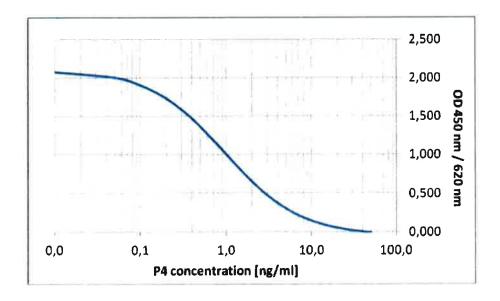
Read the OD values of the wells using a spectrophotometer at wavelength 450 nm (with a reference filter at 620 nm) and calculate the mean values. The evaluation of the ELISA can be performed using commercial ELISA evaluation software or using our EXCEL Spreadsheet which is available from Abraxis. For a manual evaluation draw a standard curve using the measured OD values on a graph paper and read the concentration for the samples from the standard curve.

## Performance characteristics

# **Assay Range & Standard Curve**

The measuring range is between 0-20 ng/ml with an analytical sensitivity of 0.06 ng/ml. A typical standard curve to be produced with this kit is shown below. This curve must not be used to

determine the values of your samples. You must run a new standard curve every time you run the assay following the quantitative protocol.



# **Specificity**

The following structurally related compounds were tested for cross reactivity of the assay.

Compound	Cross Reaction %
Progesterone	100
17-α-OH-Progesterone	< 0.1
Pregnenolone	< 0.1
Cortisol	< 0.1
Testosterone	< 0.1
17-β-Estradiol	< 0.1

### **Precision**

The intra- and inter-assay variations were analyzed using three different milk samples.

Intra-assay	sample 1	sample 2	sample 3
n	16	16	16
mean [ng/ml]	9,60	1,75	4,86
SD	0,59	0,21	0,52
CV	6%	12%	11%

Inter-assay	sample 1	sample 2	sample 3
n	8	8	8
mean [ng/ml]	9,55	1,81	4,92
SD	0,96	0,27	0,28
CV	10%	15%	6%

## Recovery

UHT milk with a fat content of 0.1% has been spiked by adding a solution with a certain progesterone concentration (different levels). The recovery in % was calculated by the ratio of the expected and the measured progesterone concentration.

Expected P4-concentration [ng/ml]	Measured P4-concentration [ng/ml]	Recovery
8,6	9,2	107%
2,8	2,9	103%
0,9	0,9	101%

# Linearity

Three different milk samples were serially diluted in UHT milk with a fat content of 0.1%. In UHT milk a progesterone concentration of 1.3 ng/ml was determined. The linearity in % was calculated by the ratio of the expected and the measured progesterone concentration.

	Dilution	Expected P4- concentration [ng/ml]	Measured P4- concentration [ng/ml]	Linearity
Sample 1	undiluted	10,40		
	1:2	6,71	5,85	115%
	1:4	5,14	4,01	128%
	1:8	3,51	3,22	109%
	1:16	2,59	2,40	108%
	1:32	1,68	1,94	86%
	1:64	1,58	1,49	106%
Sample 2	Undiluted		1,53	
	1:2	1,42	1,44	102%
	1:4	1,37	1,11	81%
	1:8	1,20	1,20	99%
Sample 3	undiluted		4,79	
	1:2	3,04	2,99	98%
	1:4	2,15	1,91	89%
	1:8	1,61	1,37	86%
	1:16	1,34	1,34	100%
	1:32	1,32	1,15	88%

# Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
В												
С												
D												
E												
F												
G												
н												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
A B	1	2	3	4	5	6	7	8	9	10	11	12
	1	2	3	4	5	6	7	8	9	10	11	12
В	1	2	3	4	5	6	7	8	9	10	11	12
В	1	2	3	4	5	6	7	8	9	10	11	12
B C D	1	2	3	4	5	6	7	8	9	10	11	12
B C D	1	2	3	4	5	6	7	8	9	10	11	12