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.

For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com

Tel.: (215) 357-3911
Fax: (215) 357-5232
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Contents

The ABRAXIS® 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 ABRAXIS® 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.

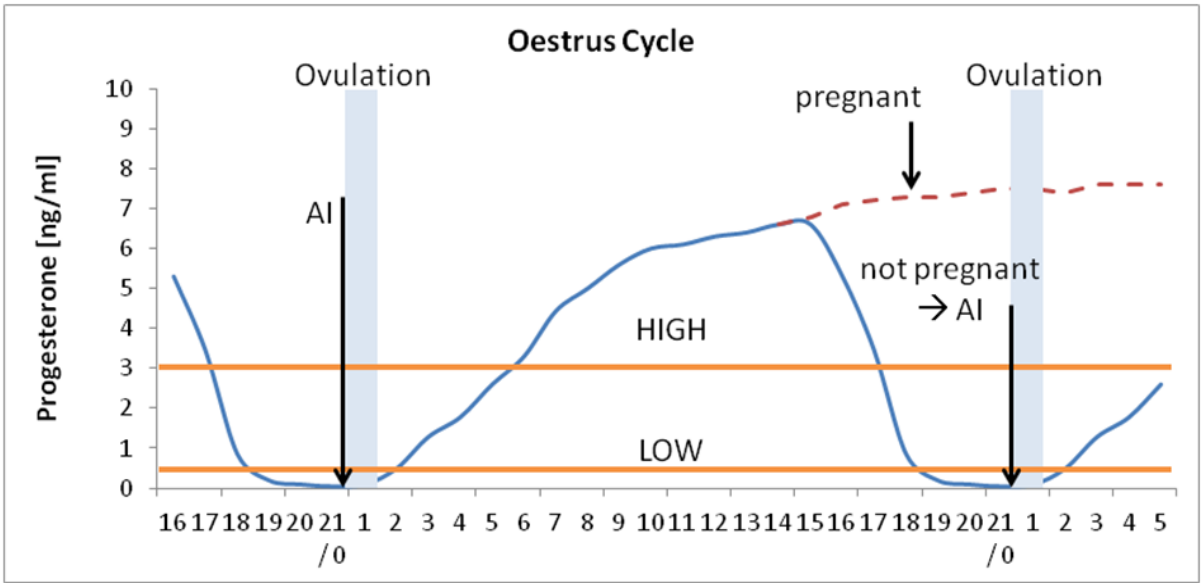


Figure 1: Progesterone levels during the oestrus cycle of a cow. AI should be performed when progesterone levels are low.

GOLD STANDARD DIAGNOSTICS

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 animal, collect a sample every 4 days. After 20-24 days, the cycle of the cow is revealed.		
Identification of silent heat or false heat	Animals not showing any heat signs during their cycle also exhibit low progesterone levels during a fertile oestrus (< LOW) and can then be used for AI. Animals showing heat signs while bearing an active corpus luteus (> LOW) cannot be used for successful AI. Retest in 4 day intervals until low progesterone levels (< LOW) indicate the oestrus.		
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 AI.
		> 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 within this period does not vary, the cow should be examined for ovarian cysts.	**< LOW** –	**<u>Follicular Cyst:</u>** Prolonged low progesterone levels can be an indication for follicular cysts.
		> LOW **+**	6 days after treatment the restart of the cycle can be monitored by elevated progesterone levels.
		> LOW **+**	**<u>Luteal Cyst:</u>** 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 too high (> 5%). Other reasons for low herd fertility can be due to e.g. stress, feeding.	**day 0** **< LOW** – **day 4** **> LOW** **+**	AI in time
		day 0 + 4 **> LOW** **+**	AI too late
		day 4 **< LOW** –	AI too early

Material

Material Supplied

1. **Microtiter plate with plate lid**: 12x8 (break apart) strips
   wells coated with a monoclonal anti-progesterone antibody
2. **Standard (1-5)**: 6 vials, 1 mL
   Concentrations 0, 0.23, 0.47, 0.94, 3.75, 20 ng/mL
3. **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 (Color) Solution**: 1 vial, 11 mL
6. **Stop Solution**: 1 vial, 6 mL
   9.9 % H_3PO_4. Avoid contact with skin and eyes!
7. **Wash Buffer (15x) Concentrate**: 1 vial, 33 mL
   Dilute before use. Content sufficient for preparation of 500 mL 1x Wash Buffer.
   See Preparation of Reagents.
8. **Instruction Booklet**

Other Essential Materials needed but not supplied

1. Deionized or distilled water
2. Precision Micropipettes for volumes of 20-200 µl and 100-1000 µl
3. Beakers for diluting the buffers
4. A microtiter plate washer (or multi-channel pipette capable of delivering 300 µl/well)
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 Buffer (15x) Concentrate:

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

The diluted 1X Wash Buffer 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 hemolytic, icteric or lipemic 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 24h at 2-8°C. For longer storage periods, the samples should be frozen at -20°C.

Test Procedure

The ABRAXIS® Progesterone ELISA can be run in two different protocols:

A quantitative protocol for up to 42 samples run in duplicates.

A semiquantitative protocol 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 quantitative protocol all standards, controls and samples should be applied in duplicates.
For the semiquantitative protocol 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 µL 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 (1X) Wash Buffer**
8. Remove the remaining liquid by patting the plate on a stack of absorbent paper towels
9. **Add 100 µL of Substrate (Color) 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

Semi-quantitative 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	-
ODs between LOW and HIGH	medium	0.47-3.75	+
ODs < HIGH (lighter blue)	high	> 3.75	++

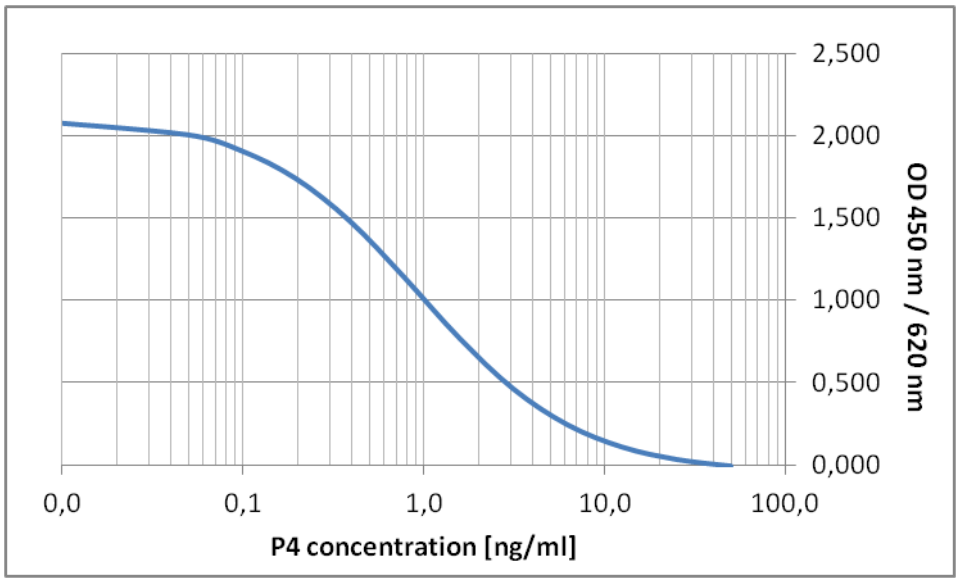
Quantitative Protocol

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 is for demonstration purposes only and not for sample interpretation. 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												
B												
C												
D												
E												
F												
G												
H												

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