RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator well have a concentration of Progesterone greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the calibrator concentration (X axis). Four-parameter curve fit can be used. If your plate reader software doesn't provide this curve fit, a spreadsheet that will perform the curve fit and sample calculation is available upon request. Report samples with concentrations less than the lowest calibrator or greater than the highest calibrator as < 0.1 ppb or > 10 ppb, respectively.

SAMPLE CALCULATIONS

Well Contents	OD	Avg. OD ± SD*	%RSD	%Bo**
Negative Control	1.886 1.857	1.871 ± 0.021	1.1	100
0.1 ppb Calibrator	1.629 1.618	1.623 ± 0.008	0.5	87
1.0 ppb Calibrator	0.831 0.851	0.841 ± 0.014	1.6	45
10.0 ppb Calibrator	0.237 0.237	0.237 ± 0.000	0.0	13

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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Beacon Analytical Systems Inc.

Progesterone Plate Kit

Cat.# 20-0242

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Progesterone Plate Kit is an immunological laboratory test for the quantitation of Progesterone in dairy products such as milk, cheese, yogurt, cream, ice cream and butter.

ASSAY PRINCIPLES

The Beacon Progesterone Plate Kit is a competitive enzyme-labeled immunoassay. Progesterone is extracted from dairy product samples such as milk, cheese, yogurt or cream, etc. The extract is diluted and tested in the immunoassay. Progesterone-enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Progesterone antibody is then pipetted into the test wells to initiate the reaction. During the 30 minute incubation period, Progesterone from the sample and Progesterone-enzyme conjugate compete for binding to Progesterone antibody which, in turn, binds to the test well. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound compounds or enzyme-labeled compounds. A clear substrate is then added to the wells and any bound Progesterone-enzyme conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of unknown samples are compared to the color of the calibrators and the Progesterone concentration of the samples is derived.

REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

- 1 frame containing 12 test strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.
- 4 vials each containing 4 mL Progesterone calibrators corresponding to 0, 0.1, 1 and 10 µg/L (ppb) Progesterone.
- 1 vial of 8 mL Progesterone-HRP Enzyme Conjugate.
- 1 vial of 8 mL Rabbit anti-Progesterone antibody.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl.)
- 1 vial containing 8 mL of 1N HCl for extraction. (Handle with care!)
- 1 vial containing 50 mL of Wash Solution concentrate (10X).

MATERIALS REQUIRED BUT NOT PROVIDED

- Methanol (ACS grade).
- Laboratory quality distilled or deionized water (DI water).
- Pipette(s) capable of dispensing 100 - 1000 µL.
- Multi-channel pipette; 8 channels capable of dispensing 50 and 100 µL. Use when running 3 or more test strips.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450 nm filter.
- Microcentrifuge and tube.
- 20 mM PBS, pH 7.2. Weigh 0.77 g of NaH2PO4.H2O (F.W. 137.99) + 2.04 g of Na2HPO4 (F.W. 141.96) + 8.5 g of NaCl then dissolve into one liter of laboratory grade water.
- Timer.

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Progesterone Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Progesterone Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use. Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
5. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
6. Use approved methodologies to confirm any positive results.
7. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

SAMPLE PREPARATION

Milk

1. Weigh 2 grams of sample in a clean vial.
2. Add 0.1 mL of 1N HCl.
3. Vortex for 1 min and stand for 10 minutes.
4. Transfer 1 mL into a microcentrifuge tube.
5. Centrifuge at 12,000 rpm for 5 minutes.
6. Discard the aqueous layer.
7. Add methanol to 1 mL.
8. Vortex for 2 minutes and try to break big particles.
9. Centrifuge for 5 minutes at 12,000 rpm.
10. Take 0.1 mL of supernatant and mix with 0.9 mL of 20 mM PBS buffer.
11. Apply **dilution factor of 10** to the concentration achieved from calibration curve.

Cheese and yogurt

1. Weigh 2 grams of sample in a clean vial
2. Add 8 mL of 0.1 N HCl*.
   (*Prepare by diluting 1N HCl 1:10 with DI water)
3. Vortex for 2 minutes and try to break big particles.
4. Transfer 1 mL into a microcentrifuge tube.
5. Centrifuge for 5 minutes at 12,000 rpm.
6. Discard the aqueous layer.
7. Fill with methanol to 1 mL.
8. Vortex for 2 minutes and try to break big particles.
9. Centrifuge for 5 minutes at 12,000 rpm.
10. Take 0.1 mL of supernatant and mix with 0.9 mL of 20 mM PBS buffer.
11. Apply **dilution factor of 50** to the concentration achieved from calibration curve.

Cream, ice cream and butter

1. Melt samples with microwave for 30 seconds or more.
2. Weigh 5 grams of sample in a 50 mL conical tube.
3. Add 20 mL of methanol and vortex for 2 minutes.
   (For butter sample, add 25 mL of methanol)
4. Transfer 1 mL into a microcentrifuge tube.
5. Centrifuge for 5 minutes at 12,000 rpm.
6. Take 0.1 mL of supernatant and mix with 0.9 mL of 20 mM PBS buffer.
7. Apply **dilution factor of 50** to the concentration achieved from calibration curve.

TEST PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Bring all kit reagents and samples to be run to room temperature.
2. Place the appropriate number of test wells into a microwell holder. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Prepare the wash solution by transferring the contents of the 10x Wash Solution concentrate to 450 mL of laboratory grade water. Swirl to mix. Transfer the diluted wash solution to a wash bottle.
4. Dispense 5**0 µL of Enzyme Conjugate** into each test well.
5. Using a pipette with disposable tips, add 5**0 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
6. Dispense 5**0 µL of Antibody Solution** into each test well.
7. Shake the plate gently to mix contents, incubate the test wells for 3**0 minutes**.
8. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with wash solution and dump. Repeat 3X for a total of four washes.
9. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the wash solution. Using a paper tower gently wipe away any remaining liquid**.**
10. Dispense **100 µL of Substrate** into each well.
11. Shake the plate gently. Incubate the wells for **30 minutes**.
12. Dispense **100 µL of Stop Solution** into each well. Shake the plate rack gently to mix.
13. Read and record the absorbance of the wells at 450 nm using a strip or plate reader.
14. Sample result must be multiplied by appropriate dilution factor.