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 β-Estradiol 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 log of the calibrator concentration (x-axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the x-axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <0.1 ppb or >5 ppb, respectively.

SAMPLE CALCULATIONS

Well Contents	OD	Avg. OD ± SD**	%RSD	%Bo*
Negative Control	1.94 1.92	1.923 ± 0.015	0.8	100
0.1ppb Calibrator	1.595 1.595	1.595 ± 0.001	0	83
0.5ppb Calibrator	1.222 1.254	1.238 ± 0.022	1.8	64
2.5 ppb Calibrator	0.612 0.605	1.595 ± 0.001	0.8	32
5 ppb Calibrator	0.44 0.448	0.444 ± 0.006	1.2	23

This data is for example purposes only.

* %Bo = (OD_{450} / 0 ppb OD_{450})*100

** Standard deviation

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.

β-Estradiol Plate Kit

Cat. # 20-0263

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The β -Estradiol Plate Kit is an immunological laboratory test for the quantitation of β -Estradiol for food safety purposes.

ASSAY PRINCIPLES

The Beacon β-Estradiol Plate Kit is a competitive enzyme-labeled immunoassay. β-Estradiol is extracted from food samples. The extract is diluted and tested in the immunoassay. β-Estradiol-enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. β-Estradiol antibody is then pipetted into the test wells to initiate the reaction. During the 30 minute incubation period, β-Estradiol from the sample and β-Estradiol-enzyme conjugate compete for binding to β-Estradiol 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 β-Estradiol-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 β-Estradiol concentration of the samples is derived. Since the same number of antibody binding sites are available in every well, and each well receives the same number of β-Estradiol-enzyme conjugate molecules, a sample containing a low concentration of β-Estradiol allows the antibody to bind many β-Estradiol-enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of β-Estradiol allows fewer β-Estradiol-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to β-Estradiol concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon β-Estradiol Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon β-Estradiol 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.
8.

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.

1 vial each of 0, 0.1, 0.5, 2.5 and 5 ppb β-Estradiol calibrators.

1 vial of β-Estradiol-HRP Enzyme Conjugate.

1 vial of Rabbit anti- β-Estradiol antibody.

1 vial containing 14 mL of Substrate.

1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Wash bottle containing deionized water.
- Pipette with disposable tips capable of dispensing 50, and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450 nm filter.
- Timer

SPECIFICITY

The β-Estradiol (E2) Plate Kit is specific for β-Estradiol (E2) analysis. The following table shows the cross reactivity pattern. %CR = 50% B0 of β-Estradiol (E2) / 50% B0 of following compounds.

Compound	%CR
β-Estradiol (E2)	100
6-Dehydroestrone	16.4
Estrone Acetate	2.8
17α-Ethynylestradiol	2.7
Estriol (E3)	0.9
Estrone 3-methyl ether	0.5
Estradiol-3-benzoate	0.0
Progesterone	< 0.01
17α-Methyl Testosterone	< 0.01
Estrone (E1)	< 0.01
Diethylstilbestrol (DES)	< 0.01
Estrone-3-Glucuronide	< 0.01

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. Dispense 5**0 µL of Enzyme Conjugate** into each test well.
4. 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.
5. Dispense 5**0 µL of Antibody Solution** into each test well.
6. Shake the plate gently to mix contents, incubate the test wells for 3**0 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with distilled water and dump. Repeat 3X for a total of four washes.
8. 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**.**
9. Dispense **100 µL of Substrate** into each well.
10. Shake the plate gently. Incubate the wells for **30 minutes**.
11. Dispense **100 µL of Stop Solution** into each well. Shake the plate rack gently to mix.
12. Read and record the absorbance of the wells at 450 nm using a strip or plate reader.
13. Multiply the concentration by the dilution factor.