
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 Estrone 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 <0.02 ppb or >0.5 ppb, respectively.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD \pm SD**	%RSD	%Bo*
Negative Control	1.948	1.985 \pm 0.003	0.1	100
	1.912			
0.02 ppb Calibrator	1.578	1.548 \pm 0.042	2.7	80
	1.518			
0.1 ppb Calibrator	0.948	0.949 \pm 0.002	0.2	49
	0.950			
0.5ppb Calibrator	0.390	0.380 \pm 0.013	3.5	20
	0.371			

This data is for example purposes only.

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

** 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.

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Estrone (E1) Plate Kit

Cat.# 20-0216

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Estrone Plate Kit is an immunological laboratory test for the quantitation of Estrone in field samples such as river water or wastewater.

ASSAY PRINCIPLES

The Beacon Estrone Plate Kit is a competitive enzyme-labeled immunoassay. Estrone is extracted from field samples such as river water or wastewater, etc. The extract is diluted and tested in the immunoassay. Estrone-enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Estrone antibody is then pipetted into the test wells to initiate the reaction. During the 30 minute incubation period, Estrone from the sample and Estrone-enzyme conjugate compete for binding to Estrone 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 Estrone-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 Estrone 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 Estrone-enzyme conjugate molecules, a sample containing a low concentration of Estrone allows the antibody to bind many Estrone-enzyme conjugate molecules. The result is a dark blue solution.

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

NOTE: Color is inversely proportional to Estrone concentration.

- Darker color = Lower concentration
- Lighter color = Higher concentration

SPECIFICITY

The Estrone (E1) Plate Kit is specific for Estrone analysis. The following table shows the cross reactivity pattern. %CR = 50% B0 of Estrone / 50% B0 of following estrogenic compounds.

Compound	%CR
Estrone (E1)	100
Estrone Acetate	206
Estrone 3-methyl ether	193
Estrone 3-(β -D-glucuronide) sodium salt	42
6-Dehydroestrone	23
Estrone 3-sulfate potassium salt	20
17 α -Ethinylestradiol	<1
β -Estradiol (E2)	<1
Estriol (E3)	<1

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Estrone Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Estrone 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 – 28°C (62 – 82°F) 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.

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 – 8°C.

1. Frame containing 12 test strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.
1. vial each of 0, 0.02, 0.1 and 0.5 ppb Estrone calibrators.
1. vial containing 10ml of 40ppb spike solution.
1. vial of Estrone-HRP Enzyme Conjugate.
1. vial of Rabbit anti- Estrone 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 μ L.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450 nm filter.
- Timer

SAMPLE PREPARATION

1. Collect 20 ml of water sample and filter through 0.2 μ m filter to remove the microorganism or particles.
2. Check the pH of the samples and make sure no acid was added as preservative.
3. Adjust the pH of the water samples between pH 5-8 with dilute acid or base before the assay.

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 **50 μ L of Enzyme Conjugate** into each test well.
4. Using a pipette with disposable tips, add **50 μ L of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
5. Dispense **50 μ L of Antibody Solution** into each test well.
6. Shake the plate gently to mix contents, incubate the test wells for **30 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with Laboratory quality distilled or deionized water and dump. Repeat 4X 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 towel gently wipe away any remaining liquid.
9. Dispense **100 μ L of Substrate** into each well.
10. Shake the plate gently. Incubate the wells for **30 minutes**.
11. Dispense **100 μ L 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.

* **Due to the limited solubility of Estrone in aqueous solutions, please bring the kit to room temperature for at least 2-4 hours before use. Warming reagents to room temperature is critical for the best assay performance.**