RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator tubes: Samples containing less color than a calibrator well have a concentration of 2, 4-D 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 <2 ppb or >100 ppb, respectively. A spreadsheet that will perform the curve fit and sample calculations can be provided upon request.

SAMPLE CALCULATIONS

Well Contents	OD_{450}	Average OD ± Std. Dev.	%RSD	%Bo*
Negative Control	1.504 1.525	1.515 ± 0.015	1.0	100.0
2.0 ppb Calibrator	1.356 1.378	1.367 ± 0.016	1.1	90.2
10 ppb Calibrator	0.887 0.892	0.890 ± 0.004	0.4	58.7
100 ppb Calibrator	0.333 0.341	0.337 ± 0.006	1.7	22.2

This data is for example purposes only.

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

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Beacon Analytical Systems Inc.

2, 4-D Tube Kit

Cat. # 20-0010

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon 2, 4-D Tube Kit is an immunological laboratory test for the quantitation of 2, 4-D residues in water in the range of 2.0 to 100 ng/mL (parts per billion or ppb). Samples containing higher concentrations can be measured by pre-dilution of the sample.

ASSAY PRINCIPLES

The Beacon 2, 4-D Tube Kit uses polyclonal antibodies that bind both 2, 4-D and a 2, 4-D -enzyme conjugate. 2, 4-D in the sample competes with 2, 4-D -enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind 2, 4-D, are immobilized to the inside of the test tubes. In the assay procedure you will:

- Add a sample or calibrator containing 2, 4-D to a test tube.
- Add 2, 4-D/enzyme conjugate. The conjugate competes with any 2, 4-D in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 20 minutes.
- Add clear substrate solution to each test tube. In the presence of bound 2, 4-D -enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every test tube, and each tube receives the same number of 2,4-D -enzyme conjugate molecules, a sample containing a low concentration of 2,4-D allows the antibody to bind many 2,4-D -enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of 2, 4-D allows fewer 2, 4-D -enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to 2, 4-D concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

SPECIFICITY

The Beacon 2, 4-D Tube Kit is specific for 2, 4-D and closely related compounds. The following table shows the % cross-reactivity versus 2, 4-D acid (used in calibrators).

Compound	% Cross-reactivity
2,4-D	100
2,4-D-methyl ester	400
2,4-DB	100
2,4-D-isopropyl ester	67
2,4-DB-butyl ester	53
2,4,5-T	9.5
MCPA	9.3
Dichlorprop	2.7
2,4,5-TP	2.2

The following list shows the compounds tested and found non-reactive at concentrations of 1,000 ppb (<0.1% cross-reactivity):

Alachlor	Aldicarb	Atrazine
Azinphos	Bromophos	Terbuthylazine
Carbofuran	Chlorpyrifos	Carbendazim
Metolachlor	Parathion	Simazine
Endothall	Dicamba	

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon 2, 4-D Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon 2, 4-D Tube 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. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. 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 – 8ºC.

2 bags each containing 20 test tubes coated with rabbit anti-2, 4-D antibodies and desiccant.

1 vial of 10 ppm 2, 4-D in calibrator stock solution.

1 vial of 2, 4-D-HRP Enzyme Conjugate.

1 vial containing 60 mL of Substrate.

1 vial containing 60 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for reading absorbance at 450 nm in 12 mm x 75mm tubes.
- Clean running water or a wash bottle containing tap or deionized water.
- Glass tubes or vials for calibrator preparation.
- 25 mL volumetric flask
- Pipet with disposable tips capable of dispensing 200 and 500 µL.
- Positive displacement pipet with disposable tips capable of delivering 250 µL.
- Paper towels or equivalent absorbent material.
- Timer

CALIBRATOR PREPARATION

The calibrator stock solution is diluted in lab grade water prior to use in the assay. Bring the stock 2, 4-D solution to room temperature prior to diluting. Diluted calibrators should be used on the day of preparation.

1. Prepare a 1:100 dilution of the 10 ppm 2, 4-D solution as follows: using a positive displacement pipet, add 250 µL of 10 ppm 2, 4-D to a clean 25 ml volumetric flask. Fill to the 25 ml mark with deionized water. Cap and invert flask 3-4 times to mix thoroughly. Label “100 ppb 2, 4-D”.
2. Dilute the 100 ppb 2, 4-D solution; in a clean vial add 1 mL 100 ppb 2, 4-D to 9 mL deionized water, label as 10 ppb 2, and 4-D.
3. In another clean vial add 0.2 mL 100 ppb 2,4-D to 9.8 ml deionized water, mix well and label as “2 ppb 2,4-D”.

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. Remove the required number of antibody coated tubes from the zip lock bag. Be sure to re-seal the bag with the desiccant to limit exposure of the tubes to moisture.
3. Pipet **500 µL of calibrators, control and samples** into the appropriate tubes. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
4. Add **200 µL of Enzyme Conjugate** to each tube.
5. Swirl the tubes rapidly to mix the contents.
6. Incubate for **20 minutes**.
7. After incubation vigorously shake the contents of the tubes into a sink. Flood the tubes completely with deionized water, and then shake to empty. Repeat this wash step three times for a total of four washes. Invert the tubes on absorbent paper and tap out as much water as possible.
8. Add **500 µL of Substrate** to each tube.
9. Incubate for **10 minutes**.
10. Add **500 µL of Stop Solution** to each tube in the same order of addition as the Substrate.
11. Read the tubes in a photometer at 450 nm. If the photometer has dual wavelength capability, read at 450 nm minus 605 or 650 nm.