RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance's to the absorbance of the calibrator tubes: Sample 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 (X axis) versus the log of the calibrator concentration (Y 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 Y 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.

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

QUALITY CONTROL

The %Bo ranges for the calibrators should fall within the following ranges:

2,4-D Calibrator (ppb)	%Bo Range
2.0	83 - 94
10	48 - 63
100	16 - 28

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 761-2199.

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.

r-Metolachlor Tube Kit

Cat.# 20-0005

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon r-Metolachlor Tube Kit is an immunological laboratory test for the quantitation of r-Metolachlor herbicide residues in water.

ASSAY PRINCIPLES

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

- Add a sample or calibrator to a test tube, followed by metolachlor-enzyme conjugate. The conjugate competes with any metolachlor 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 tube. In the presence of bound metolachlor-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 tube, and each tube receives the same number of metolachlor-enzyme conjugate molecules, a sample containing a low concentration of r-metolachlor allows the antibody to bind many metolachlor-enzyme conjugate molecules. The result is a dark blue solution.

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

NOTE: Color is inversely proportional to metolachlor concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

SPECIFICITY

The Beacon Metolachlor Tube Kit is specific for metolachlor with very slight reactivity with a limited number of closely related compounds. The following table shows the relative values for 50% Bo and the % cross reactivity versus metolachlor. All concentrations are in parts per billion (ppb).

Compound	50% B_0	% Cross Reactivity
Metolachlor	0.50	100
Acetochlor	95	0.53
Propachlor	100	0.50
Alachlor	450	0.11

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

1. Photometer for reading absorbance at 450nm in 12mm x 75mm tubes.
2. Clean running water or a wash bottle containing tap or deionized water.
3. Glass tubes or vials for calibrator preparation
4. 25 mL volumetric flask
5. Pipet with disposable tips capable of dispensing 200 and 500 µL.
6. Positive displacement pipet with disposable tips capable of delivering 250 µL.
7. Paper towels or equivalent absorbent material.
8. 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 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 1ml 100 ppb 2,4-D to 9 ml deionized water, label as 10 ppb 2,4-D. In another clean vial add 0.2 ml 100ppb 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, then shake to empty. Repeat this wash step four times for a total of five 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 450nm. If the photometer has dual wavelength capability, read at 450nm minus 605 or 650nm.