## RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: 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 (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 < 2.0 ppb or > 200 ppb, respectively.

## SAMPLE CALCULATIONS

| Well Contents | $OD_{450}$ | Average OD ± Std. Dev. | %RSD | %Bo* |
|---|---|---|---|---|
| Negative Control | 1.138<br>1.111 | 1.125 ± 0.002 | 1.7 | 100.0 |
| 2.0 ppb Calibrator | 0.977<br>0.945 | 0.961 ± 0.023 | 2.3 | 85.5 |
| 20 ppb Calibrator | 0.690<br>0.657 | 0.674 ± 0.023 | 3.5 | 59.9 |
| 200 ppb Calibrator | 0.295<br>0.279 | 0.287 ± 0.011 | 3.9 | 25.5 |

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

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Beacon Analytical Systems Inc.

# 2,4-D Plate Kit

## Cat.# 20-0011

Instructional Booklet

READ COMPLETELY BEFORE USE.

## INTENDED USE

The Beacon 2,4-D Plate Kit is an immunological laboratory test for the quantitation of 2,4-D residues in water in the range of 2.0 to 200 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 Plate 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 compounds, are immobilized to the inside of the test wells. In the assay procedure you will:

•Add a sample containing 2,4-D to a test well, followed by 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 60 minutes.

•Add clear substrate solution to each well. 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 well, and each well 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 Plate Kit is specific for 2,4-D and closely related compounds. The following table shows the % cross reactivity versus 2,4-D (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 |

## PRECAUTIONS

1. Each reagent is optimized for use in the Beacon 2,4-D Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon 2,4-D 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. 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.

1 Frame containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.

1 vial of 10 ppm 2,4-D in methanol.

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

1 vial containing 14 mL of Substrate.

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

1 Instructional Booklet

## MATERIALS REQUIRED BUT NOT PROVIDED

- Clean running water or a wash bottle containing tap or deionized water.
- Glass tubes or vials for calibrator preparation.
- Tape or Parafilm®
- Orbital shaker (optional)
- Pipet with disposable tips capable of dispensing 100 - 200µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Positive displacement pipet with disposable tips capable of delivering 200 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450 nm filter.
- 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. Label 3 clean 10 mL glass tubes or vials “2.0”, “20”, “200”.
2. Prepare a 200 ppb calibrator using the tube labeled “200”. Add 9.8 mL of lab grade water to the tube. Using a positive displacement pipet, add 200 µL of the 10 ppm stock solution. Mix thoroughly.
3. To prepare the 20 ppb calibrator, add 9.0 mL of lab grade water to the tube labeled “20”. Then, add 1.0 mL of the 200 ppb solution. Mix thoroughly.
4. To prepare the 2.0 ppb calibrator, add 9.0 mL of lab grade water to the tube labeled “2.0”. Then, add 1.0 mL of 20 ppb solution. Mix thoroughly.
4. Be sure to tightly cap the vials containing the stocks to minimize evaporative losses.

## 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 strips from the zip lock bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Pipet **150 µL of calibrators, control and samples** into the appropriate wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
4. Add **50 µL of Enzyme Conjugate** to each well.
5. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotater for continuous mixing during incubation.
6. Incubate for **60 minutes**.
7. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
8. Add **100 µL of Substrate** to each well.
9. Cover the wells and incubate for **30 minutes**.
10. Add **100 µL of Stop Solution** to each well in the same order of addition as the Substrate.
11. Read the plate on a microtiter plate reader at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
12. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4 parameter curve fit. If manual data reduction is required, proceed with next section.