PN 54004B for 2,4-D Tube Kit - Cat# ABRA-001

Intended Use

The Abraxis 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.

Test Principles

The Abraxis 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.
- Incubate this sample for 10 minutes.
- 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 10 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 Abraxis 2,4-D Tube Kit is specific for 2,4-D and closely related compounds. The following table shows the concentration required for 50% Bo and the % cross-reactivity versus 2,4-D acid (used in calibrators). Concentrations are in parts per billion (ppb).

Compound	50% Bo conc.	% Cross Reactivity
2,4-D acid	8.0	100
2,4-D methyl ester	2.0	400
2,4-DB	8.0	100
2,4-D isopropyl ester	12	67
2,4-DB butyl ester	15	53

2,4,5-T	84	9.5
MCPA	86	9.3
Dichlorprop	300	2.7
2,4,5-TP	360	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

- Store all 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).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents from kits with different lot numbers.
- Use approved methodologies to confirm any positive results.

Materials Provided in the Abraxis 2,4-D Tube Kit

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

1 vial each of 2,4-D calibrators corresponding to 0, 2.0, 10 and 100 ppb.

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

1 vial of Stop Solution

You also need these items:

- Photometer for reading absorbance at 450nm in 12mm x 75mm tubes.
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water.
- Pipet with disposable tips capable of delivering 500 µL

Assay Procedure

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. Incubate for **10 minutes** at room temperature.

4. Add **500 µL of Enzyme Conjugate** to each tube.

5. Swirl the tubes rapidly to mix the contents.

6. Incubate for **10 minutes**.

7. After incubation vigorously shake the contents of the tubes into a sink. Flood the tubes completely with cool running tap 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.

12. If the photometer 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.

Calculate Results

1. After you read all of the tubes, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

   %B° = (average OD of calibrator, control or sample x 100) ÷ average OD of negative control

2. Graph the %Bo of each calibrator on the Y (linear) axis against its diazinon concentration on the X (log) axis using semi-log graph paper. Draw the best fit line through the calibrator points.

3. Determine the 2,4-D concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.

Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Sample Calculations

Tube Contents	OD	Average OD ± SD**	%RSD	%Bo
Negative Control	1.504 1.525	1.515 ± 0.015	0.98	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 ± 0.0035	0.40	58.7
100 ppb Calibrator	0.333 0.341	0.337 ± 0.0057	1.7	22.2

Quality Control

1. 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 Abraxis products, call (215) 357-3911.

Safety

To receive complete safety information on this product, contact Abraxis, LLC. and request Material Safety Data Sheets.

2,4-D DETAILED FLOWCHART

1.

Label test tubes for Standards (Calibrators), Control, and Samples.

Tube #	Content
1, 2	Diluent/Zero Standard 0 ppb
3, 4	Standard 1, 2.0 ppb
5, 6	Standard 2, 10 ppb
7, 8	Standard 3, 100 ppb
9	Sample 1
10	Sample 2
11	Sample 3

Add 500 µL of either Standards, Control or Samples down the inside wall of each test tube by aiming the pipet tip 1/4" to 1/2" below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

2.

Add 200 µL of 2,4-D Enzyme Conjugate down the inside wall of each tube by using the technique described in Box 2. *Vortex or swirl* for 5 to 10 seconds

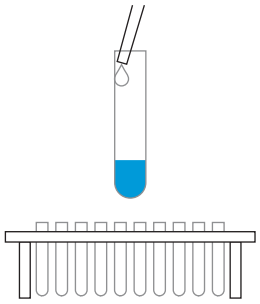
3.

React 20 minutes at room temperature (15 °- 30°C).

4.

Add 4 mL of Washing Solution to each tube (alternatively flood the tubes completely with wash solution then invert to empty tubes). Using a smooth motion, invert tubes over a sink and pour out the tube contents: keep inverted and blot the test tube rims on several layers of paper toweling.
Repeat this step 4 times.

5.

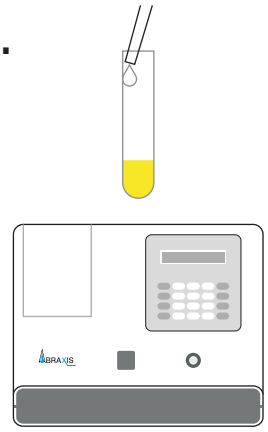


Add 500 µL of Color Reagent down the inside wall of each tube by using the technique described in Box 2.

6.

React for 20 minutes at room Temperature (15°- 30° C). During this period, add 1 mL of Washing Solution into a clean tube for use as an instrument blank in Step 8.

7.



Add 500 µL of Stopping Solution down the inside wall of each tube by using the technique previously Described. *Read* results at 450 nm within 15 minutes after adding the Stopping Solution. *Multiply* results of samples by the appropriate dilution factor (if any).

[**Safety Caution:** Stopping Solution contains diluted sulfuric acid.]

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
Web: www.abraxiskits.com

2,4-D CONCISE FLOWCHART

1.

Add 500 µL of either Standards (calibrators), Control or Samples to the bottom of each test tube.

2.

Add 200 µL of 2,4-D Enzyme Conjugate to each test tube.

Vortex or swirl.

3.

Incubate for 20 minutes.

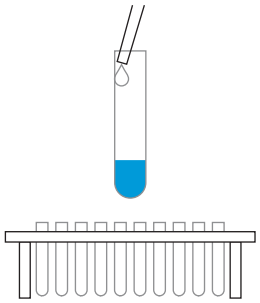
4.

Add 4 mL of Washing Solution (alternatively flood the tubes).

Invert the tube and blot.

Repeat this step 4 times.

5.



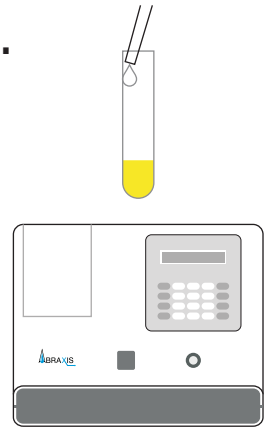
Add 500 µL of Color Reagent down the inside wall of each test tube.

6.

Incubate for 20 minutes.

Prepare blank.

7.



Add 500 µL of Stopping Solution to each test tube.

Read OD 450

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