Performance Characteristics

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-reactiveat concentrations of 1,000 ppb (<0.1% cross-reactivity):

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

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ABRAXIS® 2,4-D ELISA Tube Particle
Product No. 54004B

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 2.0to 100 ng/mL (parts per billion or ppb). Samples containing higher concentrations can be measured by pre-dilution of the sample.

Assay 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.

Materials Provided

2 Bags each containing 20 test tubes coated with rabbit anti-2,4-D antibodies and desiccant
1 vial each of 2,4-D calibrator stock solution (0, 2, 10, 100 ppb).
1 vial of 2,4-D -HRP Enzyme Conjugate
1 vial of Substrate (Color Solution)
1 vial of Stop Solution

Additional Materials (not provided with the test)

Photometer for reading absorbance at 450nm in 12mm x 75mm tubes.
Watch or timer
Wash bottle containing deionized water.
Pipet with disposable tips capable of delivering 500 µL, and 200 µL

Precautions

Store all kit components at 2°C to 8°C (36°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 last day of the month indicated bythe expiration date.
Do not mix reagents from kits with different lot numbers.
The 2,-4-D assay kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive results requiringsome action should be confirmed by an

alternative method.

Assay Procedure

Bring all kit reagents and samples to be run to room temperature. 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.

1. **Add 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.
2. **Add 200 μL of Enzyme Conjugate** to each tube. Swirl the tubes rapidly to mix the contents.
3. **Incubate for 20 minutes**.
4. After incubation, vigorously shake the contents of the tubes into a sink. **Wash the tubes completely with deionized water,** and then shake bempty. 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.
5. **Add 500 μL of Substrate (Color Solution)** to each tube.
6. **Incubate for 10-20 minutes**.
7. **Add 500 μL of Stop Solution** to each tube in the same order of addition as the Substrate.

Read the tubes in a photometer at 450nm. If the photometer has dual wavelength capability, read at 450nm minus 605 or 650nm. If the photometer has data reduction capabilities, use either a semi-log linear or 4 parameter curve fit. If manual data reductionis required, proceed with next section.

Quality Control

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, resultingin a lighter blue solution.

NOTE: Color is inversely proportional to 2,4-D concentration.
Darker color = Lower concentration Lighter color = Higher concentration
The %Bo ranges for the calibrators should fall within thefollowing ranges:

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

Calculate Results

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

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.

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