Importance of 2,4-D Determination

2,4-D is a phenoxy herbicide used in the control of broadleaf weeds. It is one of the most widely used herbicides in the world. In the United States alone, approximately 46 million pounds are used each year. 2,4-D is a selective herbicide, affecting broadleaf weeds but not grasses, making it ideal for use in both agricultural (cereal grain crops) and non-agricultural settings (residential lawns, along roadways, and in aquatic weed control in and around lakes).

Humans may be exposed to 2,4-D through direct physical contact with treated plants or water sources or thorough ingestion of contaminated foods or drinking water. Drinking water sources can be contaminated through run-off (surface water) or leaching (ground water). 2,4-D has been found in surface waters across the U.S. The U.S. EPA lists 2,4-D as a compound which is likely to leach from soil and it has been found in groundwater in the U.S. as well as in Canada. Human exposure to 2,4-D can cause liver toxicity at low doses, producing inflammation, jaundice, and even acute hepatitis. Golfers exposed to 2,4-D have shown permanent liver damage leading to cirrhosis. Damage may also occur to the kidneys and adrenals, ovaries, testes, and thyroid. 2,4-D can cause neurotoxicity and has been linked to an increased risk of amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's Disease).

The EPA, under the Safe Drinking Water Act, has established a maximum contaminant level (MCL) of 70 ppb in drinking water. The World Health Organization has established an Acceptable Daily Intake (ADI) of 0.01 mg/kg of body weight.

The ABRAXIS® 2,4-D ELISA allows for the analysis of 41 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 2 hours.

Performance Data

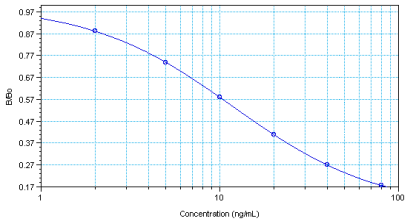
Test sensitivity: The limit of quantitation for 2,4-D (90% B/B_0 calculated from the average of 28 calibration curves) is approximately 1.67 ng/mL. The middle of the test (50% B/B_0 calculated from the average of 28 calibration curves) is approximately 13.71 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: The standard curve R^2 must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples should be ≤15%. The Standard 0 absorbance value should be between 0.8 - 3.000.

Specificity: Cross-reactivity of the ABRAXIS® 2,4-D ELISA for related herbicides:

2,4-D	100%
2,4,5-T	66%
2,4-D isopropyl ester	57%
2,4-D methyl ester	56%
2,4-DB butyl ester	26%
MCPA	13%
2,4-DB	2.5%
Dichlorprop	1.2%
2,4,5-TP	< 0.1%

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

General Limited Warranty: Gold Standard Diagnostics warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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ABRAXIS® 2,4-D ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of 2,4-D in Water Samples

Product No. 54003A

1. General Description

The ABRAXIS® 2,4-D ELISA is an immunoassay for the quantitative and sensitive screening of 2,4-D. This test is suitable for the quantitative and/or qualitative screening of 2,4-D in water samples. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of 2,4-D. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The ABRAXIS® 2,4-D ELISA should be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the end of the month indicated by the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of 2,4-D by specific antibodies. 2,4-D, when present in a sample, and a 2,4-D-HRP analogue compete for the binding sites of the mouse anti-2,4-D antibodies in solution. The 2,4-D antibodies are then bound by a second antibody (goat anti-mouse) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of 2,4-D present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® 2,4-D ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

Each reagent is optimized for use in the ABRAXIS® 2,4-D ELISA kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ABRAXIS® 2,4-D ELISA kits with different lot numbers.

The ABRAXIS® 2,4-D ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. 2,4-D Standards (7): 0, 2, 5, 10, 20, 40, 80 ng/mL (ppb), 1 mL each
3. Antibody Solution (mouse anti-2,4-D), 6 mL
4. 2,4-D-HRP Conjugate Solution, 6 mL
5. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
6. Sample Diluent, 25 mL
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section C)
5. Deionized or distilled water
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 6: Standards
(0; 2; 5; 10; 20; 40; 80 ppb)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Std 6										
F	Std 2	Std 6										
G	Std 3	Samp1										
H	Std 3	Samp1										

F. Assay Procedure

1. **Add 50 µL of the standards or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. **Add 50 µL of enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. **Add 50 µL of antibody solution** to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
4. **Incubate the strips for 60 minutes at room temperature**.
5. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips three times using the diluted wash buffer.** Use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. **Add 150 µL of substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from direct sunlight.
7. **Add 100 µL of stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding 2,4-D concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb (or ng/mL) of 2,4-D by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Gold Standard Diagnostics upon request.

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of 2,4-D than standard 1 (2 ppb) should be reported as containing < 2 ppb of 2,4-D. Samples showing a higher concentration than standard 6 (80 ppb) must be diluted further with the provided sample diluent and re-analyzed.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of 2,4-D greater than the concentration of that standard. Samples which have higher absorbances than a standard will have concentrations of 2,4-D less than that standard.

As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

H. Recoveries

Surface water samples from various sources were spiked with various levels of 2,4-D and assayed using the ABRAXIS® 2,4-D plate ELISA. The following results were obtained:

2,4-D Added (ppb)	Mean Recovery (ppb)	S.D. (ppb)	% Recovery
3.75	3.808	0.563	102%
7.5	7.480	0.679	100%
15	14.228	1.168	95%
30	28.752	3.124	96%
Average			98%