ABRAXIS® Dicamba ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Dicamba in Water, Durum Wheat, and Soil

Product No. 500050

1. General Description

The ABRAXIS® Dicamba ELISA is an immunoassay for the detection of Dicamba. This test is suitable for the quantitative and/or qualitative detection of Dicamba in water, durum wheat, and soil. If necessary, positive samples should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

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

3. Storage and Stability

The ABRAXIS® Dicamba ELISA kit should be stored in the refrigerator (2-8°C). All components of the kit must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for the proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Dicamba by specific antibodies. The standards, control, and samples are added to microtiter wells coated with polyclonal anti-Dicamba antibodies, followed by Dicamba enzyme conjugate. A competitive reaction occurs between the Dicamba (which may be present in the sample) and the enzyme labelled Dicamba competes for the binding sites of the anti-Dicamba antibodies immobilized on the microtiter plate. The reaction is allowed to incubate for 60 minutes, followed by a washing step. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Dicamba present in the sample. The color reaction is stopped after a specified time and is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run (**see Section H. Evaluation**).

5. Limitations of the Dicamba ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded. The presence of the following substances were found to have no significant effect on the ABRAXIS® Dicamba ELISA results: **up to 10,000 ppm** - sodium chloride, manganese, potassium, phosphate, sulfate, calcium, magnesium; **up to 1,000 ppm** - zinc, sodium thiosulfate, nitrate; **up to 100 ppm** - iron, ascorbic acid, silicofluoride; **up to 10 ppm** - copper; **up to 1 ppm** - humic acid, sodium chlorite. If solvents were used for extractions, solvent concentrations (methanol, acetonitrile, dimethyl sulfoxide) should be less than 1% in assay.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit (or reagents), 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 during the test performance (lower than 10°C or higher than 30°C).

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

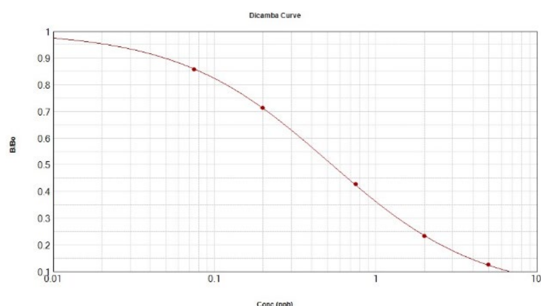
6. Working Instructions

A. Materials Provided

1. Microtiter plate coated anti-Dicamba antibody, in a resealable foil pouch with desiccant.
2. Dicamba Standards (6): 0.0, 0.075, 0.200, 0.750, 2.000, and 5.000 ppb.
3. Dicamba Control (1): 0.500 ± 0.125 ppb.
4. Dicamba Sample Diluent, 2 x 30 mL.
5. Dicamba HRP Conjugate Solution, 6.5 mL
6. Wash Solution (5X) Concentrate, 100 mL
7. Color (Substrate) Solution (TMB),18.0 mL
8. Stop Solution,13.5 mL

Performance Data

Test Sensitivity: The Dicamba ELISA has an estimated detection limit (90% B/B0) of 0.050 ppb. The middle of the test (50% B/B0) is approximately 0.550 ppb. Determinations closer to the middle of the calibration range of the test yield the most accurate results. The limit of quantification (LOQ) is 0.075 ppb in water and 7.5 ppb in durum wheat and soil.



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

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 and Control should be ≤15%. The Control should be within its acceptable range and Standard 0 absorbance value should be between 0.8 - 3.000.

Cross-reactivities:

Compound:	LDD ppb	50% (ppb)
Dicamba	0.053	0.630
5-hydroxydicamba	0.030	0.340
3,6 Dichloro-2-hydroxy Benzoic Acid	1.9	34.0
Silvex/Fenoprop	15.1	320.0
2-Chlorobenzoic Acid	31.3	490.0
Dichlorprop	48.7	1320.0
Triclopyr	550	2800.0
2,4,5-T	1000	N/A
Mecoprop	5000	N/A
Benzoic Acid	10000	N/A
4-Chloro-o-Tolyloxyacetic Acid	10000	N/A
3,5-Dichlorosalicylic Acid	10000	N/A
Dinoseb	10000	N/A
3-Phenoxybenzoic Acid	10000	N/A
Isozaben	>1000	N/A
Trifluralin	>1000	N/A
Atrazine	>10000	N/A
Dalapon	>10000	N/A
2,4-D (2,4-dichlorophenylacetic	>10000	N/A
Nonylphenol	>10000	N/A
Glyphosate	>100000	N/A
Glufosinate	>100000	N/A
AMPA	>100000	N/A

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

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Disposable serological pipettes, 2.0, 5.0, 10 mL
4. Microtiter plate reader with wavelength 450 nm
5. Timer
6. Large sized bottles (500 mL sized or larger)
7. Parafilm or adhesive film microplate cover
8. 15 mL and 2 mL plastic centrifuge tube or equivalent
9. 4 mL glass vials with Teflon caps or 12 x 75 mm borosilicate glass tubes
10. 0.2µm Cellulose Acetate syringe filters
11. 10 mL or higher syringes
12. Deionized water
13. Vortex mixer/shaker and rotator
14. Microcentrifuge capable of 8 100 x g or 10 000 rpm
15. Analytical balance, 2 decimal place (weigh range ± 0.05 grams)

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a repeater/stepping pipette for adding the conjugate, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please use only 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 foil bag. The remaining strips should be stored in the foil bag and zip-locked closed.
3. The Standards, Control, HRP conjugate, color substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized water to 100 mL wash buffer (5X) concentrate into a large sized clean bottle.
5. The stop solution should be handled with care as it contains diluted sulfuric acid (H_2SO_4).

D. Sample Collection and Handling

Collect water samples, from ponds/streams, in glass sample containers. Samples containing gross particulate matter should be filtered prior to analysis using any of the following syringe filters: Environmental Express 0.2 mm Cellulose Acetate (PN SF020CA-CP). Store samples refrigerated for up to 1 week. For storage periods greater than 2 weeks, samples should be stored frozen. Chlorinated/Tap water was not evaluated using this test.

E. Preparation of Samples

Samples should be analyzed immediately after preparation, if possible, or can be stored 2-8°C for 5 days or -20°C indefinitely. Please inquiry about preparation of samples for other matrices.

Durum wheat and Soil Samples

1. Weigh 1.00 ± 0.05 grams of sample into a 15 mL plastic centrifuge tube.
2. Add 10.0 mL of deionized water, vortex thoroughly for 10 seconds. Mix using a rotator for 10 minutes.
3. Let sample settle for >2 minutes.
4. Add 2.0 mL of sample to 2 mL microcentrifuge vial. Centrifuge for 5 min at 8,100 X g or 10,000 rpm in microcentrifuge. Remove and transfer supernatant into a clean glass vial. Optional: Use syringe filter with 0.2µm Cellulose Acetate filter if microcentrifuge is unavailable.
5. Dilute supernatant 10-fold by adding 100 µL of supernatant/filtrate to 900 µL Dicamba Sample Diluent in 4 mL glass vial or 12 x 75 mm borosilicate glass tube. Vortex to mix.
6. Proceed to Section G. Assay Procedure, step 1. The Dicamba concentration contained in sample is then determined by multiplying the ELISA result by the dilution factor of 100.

F. Working Scheme

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

	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	Control										
F	Std 2	Control										
G	Std 3	Samp1										
H	Std 3	Samp1										

Std 0-Std 5: Standards
0.0; 0.075; 0.200; 0.750; 2.000; 5.000 ppb
Control
Samp1, Samp2, etc.: Samples

G. Assay Procedure

1. **Add 100 µL of the standards, control, and samples** to the microtiter wells according to the working scheme given. Analysis of the standards in duplicate is recommended.
2. **Add 50 µL of the Dicamba HRP Conjugate 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 plate in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
3. **Incubate for 60 minutes at room temperature**.
4. Remove the covering and decant the content of the wells into a sink. **Wash the microtiter plate three (3) times** using 1X Diluted Wash Buffer. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted microtiter plate dry on a stack of paper towels.
5. **Add 150 µL of color (substrate) solution** to each individual well. Cover the wells with parafilm or tape and mix the contents by moving the plate in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. **Incubate the strips for 20 minutes at room temperature**. Protect the strips from direct sunlight.
6. **Add 100 µL of stop solution** to the wells in the same sequence as for the substrate solution.
7. Read the absorbance at 450 nm with a 630 nm blank using a microplate ELISA photometer within 15 minutes after the addition of stop solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs [4-Parameter – if not available, please contact GSD]. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 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/B0 for each standard on the vertical linear (y) axis versus the corresponding Dicamba concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb of Dicamba by interpolation using the standard curve.

For durum wheat and soil samples, the ELISA results must be multiplied by a factor of 100 to account for the necessary dilution. Samples showing a concentration lower than Standard 1 (0.075 ppb) should be reported as < 7.50 ppb of Dicamba. Samples showing a higher concentration than Standard 5 (5.0 ppb) can be reported as > 500.00 ppb or diluted further and re-analyzed to obtain an accurate quantitative result.