Dicamba is a selective systemic herbicide used to control annual, biennial, and perennial broadleaf weeds in a variety of food and feed crops as well as in non-agricultural settings. It has come under significant scrutiny due to its tendency to spread from treated fields into neighboring fields, causing damage.

## ABRAXIS® DICAMBA ELISA TEST KIT VALIDATION

Validations have been completed to ensure that the Gold Standard Diagnostics' ABRAXIS® Dicamba ELISA 96 well test kits are accurate, specific, reproducible, and rugged. Test kit validation assures reliability during normal use and is the process of providing documented evidence that the method does what it is intended to do. The performance results below demonstrate:

- Sensitivity (Limit of Detection)
- Specificity
- Limit of Quantitation
- Lot-to-lot Variability
- Precision

## DICAMBA TEST METHOD

The test is a direct competitive ELISA based on the recognition of Dicamba by specific polyclonal antibodies.
The Dicamba (which may be present in the sample) and the enzyme labelled Dicamba compete for the binding sites on the anti-Dicamba antibodies immobilized on the microtiter plate. After the 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. This method allows for the detection of Dicamba between 0.075 to 5 ppb in water and 7.5 to 500 ppb in soil and durum wheat including sample preparation, can be performed and results obtained in less than 2 hours.

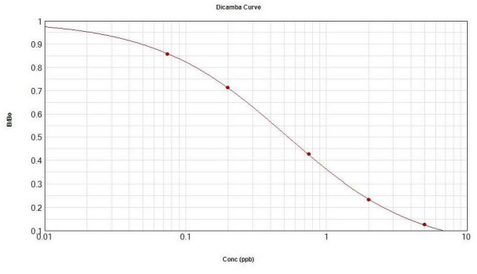
## SAMPLE MATRICES

For the performance validation studies, extracts from these various sample matrices were evaluated for Dicamba using the ABRAXIS® Dicamba ELISA test method.

- Water
- Soil
- Durum Wheat

## SENSITIVITY (LOD)

The ABRAXIS® Dicamba ELISA has a limit of detection (LOD) (90% B/B0) of 0.050 ppb in water and 5.00 ppb in soil and durum wheat, compensating for matrix dilution. The middle of the test (50% B/B0) is approximately 0.75 ppb in water and 75.0 ppb in soil and durum wheat. Determinations closer to the middle of the calibration curve give the most accurate results.




Curve Fit : 4-Parameter Logistic $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

| | Parameter | Estimated Value | Std. Error | Confidence Interval |
|---|---|---|---|---|
| StdCurve<br>$R^2$ = 1.000<br>EC50 = 0.518 | A | 0.999 | 0.002 | [0.990, 1.009] |
| | B | 0.928 | 0.011 | [0.880, 0.976] |
| | C | 0.518 | 0.010 | [0.478, 0.559] |
| | D | 0.017 | 0.006 | [-0.008, 0.042] |

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for control and samples: <15%

## SPECIFICITY

The cross-reactivity of the ABRAXIS® Dicamba ELISA for related analogs and various common herbicides expressed as the least detectable dose (LDD) or 90% B/B0 and as require for 50% inhibition (50% B/B0) are shown below.

| 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 acid) | >10000 | N/A |
| Nonylphenol | >10000 | N/A |
| Glyphosate | >100000 | N/A |
| Glufosinate | >100000 | N/A |
| AMPA | >100000 | N/A |

## LIMIT OF QUANTITATION (LOQ)

Validated LOQ values were determined by spiking gravimetric dicamba into a residue matrix (Durum Wheat and Soil) to approximate these concentrations. At least ten replicate test portions were analyzed using the ELISA method.

**Results:** All the 10 ppb samples were detected with a %CV of less than 10%.

Durum Wheat (DW) #055929 (#22) LC/MS < 10 ppb

| | 10ppb | 10ppb |
|---|---|---|
| (n) | DW | 5pCk |
| 1 | 11.4 | 8.9 |
| 2 | 13.3 | 10.0 |
| 3 | 11.8 | 10.4 |
| 4 | 12.2 | 9.1 |
| 5 | 12.7 | 8.8 |
| 6 | 13.9 | 9.9 |
| 7 | 12.7 | 9.3 |
| 8 | 13.4 | 8.6 |
| 9 | 12.4 | 10.2 |
| 10 | 14.1 | 11.1 |
| 11 | 12.7 | 8.9 |
| 12 | 12.2 | 9.7 |
| 13 | 12.9 | 9.1 |
| 14 | 14.1 | 9.3 |
| 15 | 13.5 | 9.1 |
| 16 | 15.3 | 8.7 |
| 17 | 11.9 | 10.0 |
| 18 | 13.8 | 10.8 |
| 19 | 13.7 | 9.8 |
| 20 | 11.3 | 10.1 |
| **Avg ppb** | **12.9** | **9.6** |
| Stdev | 1.025 | 0.723 |
| **%CV** | **7.9** | **7.5** |
| **%recovery** | **129.5** | **95.9** |

ABXSoil LC/MS < 10 ppb

| | l0ppb | l0ppb |
|---|---|---|
| (n) | Soil | SpCk |
| 1 | 11.3 | 11.4 |
| 2 | 10.2 | 11.3 |
| 3 | 10.2 | 11.7 |
| 4 | 9.0 | 10.5 |
| 5 | 11.8 | 11.7 |
| 6 | 10.7 | 12.1 |
| 7 | 10.8 | 10.9 |
| 8 | 10.6 | 10.5 |
| 9 | 12.3 | 12.1 |
| 10 | 10.8 | 12.3 |
| 11 | 11.1 | 10.3 |
| 12 | 10.8 | 11.2 |
| 13 | 11.9 | 12.6 |
| 14 | 11.6 | 12.0 |
| 15 | 10.3 | 10.9 |
| 16 | 11.9 | 11.6 |
| 17 | 11.7 | 12.4 |
| 18 | 12.4 | 12.7 |
| 19 | 12.5 | 12.6 |
| 20 | 10.8 | 12.3 |
| **Avgppb** | **11.1** | **11.7** |
| Stdev | 0.875 | 0.764 |
| **%CV** | **7.9** | **6.6** |
| **%recovery** | **111.2** | **116.6** |

## LOT-TO-LOT VARIATION

Lot-to-lot variation is a frequent challenge that limits a user or laboratory's ability to produce consistent results over time. Assuring lot-to-lot consistency is important to a successful testing program.

Water, Durum Wheat, and Soil spiked samples were tested alongside Dicamba standards and control to evaluate product consistency through quantitation in two different ELISA test kit lots. All samples, standards, and controls were analyzed in duplicates per the kit instructions.

**Results:** The concentration and % Recoveries for all the spiked samples are consistent between the two kit lots showing excellent reproducibility.

| Kit Lot 2 (2410902158) | | |
|---|---|---|
| Spiked Samples | Conc ppb | %Recovery |
| Water-Low | 12.3 | 82.3 |
| Water-Mid | 58.9 | 117.8 |
| Water-High | 330.0 | 110.0 |
| Durum Wheat-Low | 14.9 | 99.4 |
| Durum Wheat-Mid | 45.4 | 90.8 |
| Durum Wheat-High | 298.1 | 99.4 |
| Soil-Low | 11.6 | 77.1 |
| Soil-Mid | 45.2 | 90.5 |
| Soil-High | 305.2 | 101.7 |

| Kit Lot 3 (2419302668) | | |
|---|---|---|
| Spiked Samples | Conc ppb | %Recovery |
| Water-Low | 14.5 | 96.5 |
| Water-Mid | 55.0 | 110.0 |
| Water-High | 364.3 | 121.4 |
| Durum Wheat-Low | 16.6 | 110.5 |
| Durum Wheat-Mid | 52.9 | 105.8 |
| Durum Wheat-High | 318.6 | 106.2 |
| Soil-Low | 14.6 | 97.5 |
| Soil-Mid | 57.6 | 115.1 |
| Soil-High | 304.7 | 101.6 |

## PRECISION STUDY

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility.

Intermediate precision and reproducibility were determined by spiking deionized Water, Soil, and two Durum Wheat samples at three different concentrations that were then analyzed using the ELISA method.

**Results**: The inter- and intra-assay %CVs for each of the three concentrations in each of the sample matrices are consistent, showing excellent precision.

diH2O Spikes CV% average between (inter) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Mean | 0.147 | 0.514 | 3.126 |
| StdDev. | 0.010 | 0.012 | 0.107 |
| CV% | 7.1 | 2.4 | 3.4 |

CV% average within (intra) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Average | 0.147 | 0.514 | 3.126 |
| % Recovery | 97.8 | 102.9 | 104.2 |
| Std. Dev. | 0.013 | 0.022 | 0.129 |
| % CV | 9.2 | 4.2 | 4.1 |

Soil Spikes CV% average between (inter) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Mean | 0.137 | 0.481 | 2.884 |
| StdDev. | 0.009 | 0.021 | 0.061 |
| CV% | 6.2 | 4.4 | 2.1 |

CV% average within (intra) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Average | 0.137 | 0.481 | 2.884 |
| % Recovery | 91.5 | 96.1 | 96.1 |
| Std. Dev. | 0.012 | 0.026 | 0.085 |
| % CV | 8.7 | 5.5 | 2.9 |

Durum Wheat Spikes CV% average between (inter) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Mean | 0.158 | 0.492 | 2.897 |
| StdDev. | 0.011 | 0.009 | 0.071 |
| CV% | 7.0 | 1.9 | 2.4 |

CV% average within (intra) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Average | 0.158 | 0.492 | 2.897 |
| % Recovery | 105.4 | 98.4 | 96.6 |
| Std. Dev. | 0.014 | 0.023 | 0.138 |
| % CV | 9.1 | 4.7 | 4.8 |

Durum Wheat Spikes CV% average between (inter) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Mean | 0.156 | 0.500 | 2.952 |
| StdDev. | 0.018 | 0.034 | 0.104 |
| CV% | 11.3 | 6.8 | 3.5 |

CV% average within (intra) assay

| ppb | 0.150 | 0.500 | 3.000 |
|---|---|---|---|
| Average | 0.156 | 0.500 | 2.952 |
| % Recovery | 103.8 | 99.9 | 98.4 |
| Std. Dev. | 0.021 | 0.042 | 0.133 |
| % CV | 13.3 | 8.4 | 4.5 |

## CORRELATION WITH INSTRUMENTAL ANALYSIS

The aim of the correlation study is to assess the closeness of agreement between the results of the ELISA Kit and LC-MS/MS methods for the determination of Dicamba in water and grains.

The study was performed to evaluate the amount of Dicamba in different matrix samples by running each sample using the ELISA Test Kit and LC-MS/MS.

**Results:** The data shows a good correlation between ELISA and LC-MS/MS performance. All the samples below the ELISA LOD (0.075 ppb in water and 7.5 ppb in Durum Wheat and Soil) that would be reported as Non-Detect were confirmed with LC-MS/MS. The samples with Dicamba present were confirmed by LC-MS/MS with excellent correlation, indicated by recoveries between 80% and 120%, attesting that instrumental analysis and ELISA are not significantly different.

Water Samples

| | ELISA ppb | LC-MS/MS ppb | % recovery |
|---|---|---|---|
| 1 | 0.12 | <0.10 | |
| 2 | 0.17 | <0.10 | |
| 3 | 0.10 | <0.10 | |
| 4 | <0.50 | <0.10 | |
| 5 | 2.54 | 2.60 | 97.7 |
| 6 | 2.64 | 2.60 | 102 |
| 7 | 2.48 | 2.60 | 94.6 |
| 8 | 2.44 | 2.60 | 94.6 |

Durum Wheat Samples

| | ELISA ppb | LC-MS/MS ppb | % recovery |
|---|---|---|---|
| 1 | <5.00 | <10.00 | |
| 2 | <10.00 | <10.00 | |
| 3 | 43.70 | 40 | 109 |
| 4 | 46.41 | 40 | 116 |

Soil Samples

| | ELISA ppb | LC-MS/MS ppb | % recovery |
|---|---|---|---|
| 1 | <5.00 | <10.00 | |
| 2 | <10.00 | <10.00 | |
| 3 | <10.00 | <10.00 | |

## MANUAL / AUTOMATED TEST PROCEDURE

Dicamba ELISA tests can be run manually with laboratory equipment that includes pipettors and a microplate reader among others. The test method can also be automated with the BOLT or ThunderBolt automated ELISA analyzers. These analyzers will add and dispense reagents, wash, incubate, shake, and interpret results with a built-in microplate reader, displaying them on an on-board computer. Contact Gold Standard Diagnostics for information on automated analyzers.

| Part Number | Product Description |
|---|---|
| 500050 | ABRAXIS® Dicamba ELISA 96-test |

GOLD STANDARD DIAGNOSTICS

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