Importance of Diuron Determination

One of the most frequently used herbicides is Diuron; it belongs to the class of phenylurea herbicides. They are applied for weed control e.g. on railway lines, roads, parking lots or industrial areas as well as for algae control in fishponds. It is desirable to check water samples for possible residues of Diuron as this herbicide may frequently occur in water and soil.

The ABRAXIS® Diuron ELISA allows the determination of 40 samples in duplicates. Only a few mL of sample are required. The test can be performed in less than 1 hour.

Performance data

Test sensitivity: The detection limit for Diuron is 0.03 µg/L (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B_0) is at 0.25 µg/L. Determinations close to the middle of the tests yield 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.

Selectivity: The ELISA for Diuron recognizes also Linuron, Chlorbromuron and Neburon (CR: >10%).

Cross-reactivities:

Diuron	100% (per definition)
Neburon	1250 %
Chlorbromuron	62.5 %
Linuron	25 %
Chlortoluron	7.8 %
Propanil	4.8 %
Monuron	<1 %
Monolinuron	<1 %
Fenuron	<1 %
Bromuron	<1 %
Isoproturon	<1 %
Propham	<1 %

Cross-reactivities with herbicides different from phenylureas have not been observed.

Samples: Drinking water, ground water and surface water samples were tested for matrix effects in the ELISA. No matrix effects were determined.

Recovery: Spiking of samples with different concentrations of Diuron (0.05 - 3 µg/L) yielded a recovery of 80-110%.

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ABRAXIS® Diuron ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Diuron in Water Samples

Product No. 520001

1. General Description

The ABRAXIS® Diuron ELISA is an immunoassay for the sensitive determination of Diuron, a phenylurea herbicide. This test is suitable for the determination of Diuron in water samples. A previous sample preparation is not required. If required, positive samples can be analyzed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions of the test kit contain the herbicide Diuron. In addition to this, the substrate solution contains tetramethylbenzidine and the stop solution sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

3. Storage and Stability

The ABRAXIS® Diuron ELISA has to be stored in the refrigerator (2-8°C). The solutions have to be adjusted to room temperature (20-25°C) before use of the test kit. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

The test is based on the recognition of Diuron by specific antibodies. Diuron present in the sample and a phenylurea-enzyme conjugate compete for the binding sites of the antibodies immobilized on the plate. 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 Diuron present in the sample. The color reaction is stopped after 20 min and the color is evaluated using an ELISA reader.

5. Limitations of the ABRAXIS® Diuron ELISA, Possible Test Interference

Water samples may contain a number of various ingredients. Due to the high variability of possible ingredients, test interference caused by matrix effects cannot be completely excluded. Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, wrong sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures during the test performance (lower than 15°C or higher than 30°C).

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with anti-Diuron antibody. Twelve strips of 8 detachable wells
2. Standard Diluent, 25mL
3. Assay Buffer, 6.5 Ml
4. Standards (6), containing Diuron concentrations of: 0.0, 0.03, 0.1, 0.3, 1.0, 3.0 ppb, 1 mL
5. Enzyme Conjugate, 6.5 mL
6. Negative Control, concentration <0.03 ppb
7. Positive Control, concentration 0.3 ppb
8. ABRAXIS® Wash Solution 5X Concentrate, 100 mL
9. Substrate/Color Solution, 13.5 mL
10. Stop Solution, 6.5 mL

B. Additional Material (not included with the test kit)

1. Micro-pipets with disposable plastic tips (10-100 µL, 100-1000 µL)
2. Multi-channel pipet (10-200 µL) or stepper pipet with plastic tips (10-200 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wavelength 450 nm
5. Shaker for microtiter plates

C. Test Preparation

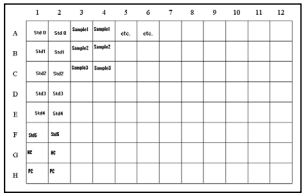
Micro-pipetting equipment and the matching pipet tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipet or a stepping pipet for adding the enzyme tracer, the substrate solution and the stop solution in order to equalize the incubation periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted to each other.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. The washing buffer concentrate (5x concentrated) has to be diluted 5-fold to the amount required with distilled H_20 (i.e. 100 mL of wash buffer 5X and 400 mL of DI water). The number of microtiter plate strips required is removed from the aluminum foil. The remaining strips are stored back in the aluminum foil and closed again using the white plastic clip. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, positive and negative controls, enzyme tracer, substrate and stop solution are ready to use and do not require any further dilutions.
4. The stop solution has to be handled with care as it contains diluted sulfuric acid.

D. Working Scheme

The microtiter plate consists of 12 X 8 strips, which can be used individually for the test. The standards have to be applied in each test. Do not use the values of standards, which have been determined in a test performed previously

St0-St5: Standards
(0, 0.03, 0.10, 0.3, 1.0, 3.0, ug/L)
NC (Negative Control): <0.03 µg/L
PC (Positive Control): 0.30 µg/L
Sa1, Sa2, Sa3, etc.: Samples



B. Assay Procedure

1. **Add 25 µL of the assay buffer** into each individual well using a multi-channel or stepping pipet.
2. **Add 50 µL of the standard solutions, the controls or the samples** into the wells of the individual test strips according to the working scheme given. We recommend using duplicates or triplicates.
3. **Add 50 µL of enzyme conjugate solutions** to the individual wells successively using a multi-channel pipet or a stepping pipet.
4. **Incubate the strips for 30 min at room temperature** (if possible use an orbital shaker).
5. **Wash the strips three times using the washing buffer solution.** Please use at least a volume of 300 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stock of paper.
6. **Add 100 µL of substrate solution** to the wells.
7. **Incubate the strips for 25-30 min at room temperature in the darkness**, if possible on a shaker. Protect the strips from light.
8. **Add 50 µL of stop solution** to the wells in the same sequence as for the substrate solution.
9. Read results at 450 nm using an ELISA photometer.

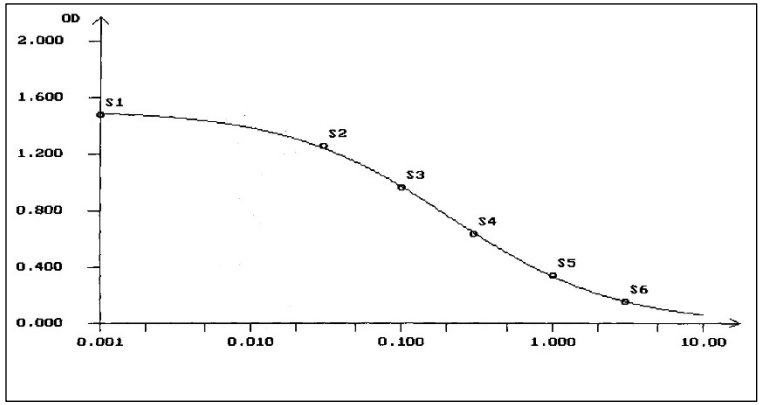
C. Evaluation

The evaluation of the ELISA can be Performed using commercial ELISA evaluation programs (Logit/Log or 4-Parameter). 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 the Zero Standard (Standard 0). Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Diuron concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of Diuron by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing a lower concentrations of Diuron compared to standard 1 (0.03 µg/L) are considered as negative. Samples showing a higher concentration than standard 5 (3 µg/L) must be diluted further to obtain more accurate results. The concentration of the negative and positive controls should be in the range given in the test instructions (±20%).

F. Standard Curve

(These values are used for demonstration purposes; do not use these values for your determinations)



G. References

1. P. Schneider, M.H. Goodrow, S.J. Gee, B.D. Hammock, A highly sensitive and rapid ELISA for the arylurea herbicides Diuron, Monuron and Linuron. J. Agric. Food Chem. 42, 1994, 413-422. (2)
2. B. Hock, T. Giersch, A. Dankwardt, K. Kramer, S. Pullen, Toxicity Assessment and On-line monitoring: Immunoassays, Environ. Toxicol. Water Qual. 9, 1994, 243-262.