Importance of Metolachlor Determination

Metolachlor is one of the most widely used herbicides in agriculture throughout the world. It is a pre-emergence herbicide used on a variety of crops including corn, soybeans, peanuts and cotton. An aniline derivative and member of the chloracetanilide family of herbicides, Metolachlor was originally produced as a mixture of the S- and R-isomers (only the S-isomer is active). Modern production methods produce only the S-isomer. Metolachlor may be used alone or in combination with other herbicides. Metolachlor readily leaches into run-off and has been found in both surface and ground waters.

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

Performance Data

Test sensitivity: The limit of detection for Metolachlor, calculated as 90% B/Bound, is approximately 0.058 ng/mL The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 1.6 ng/mL. Determinations closer to the middle of the calibration range of the test 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 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.

Recoveries: Five (5) groundwater samples were spiked with various levels of Metolachlor and assayed using the ABRAXIS® Metolachlor Plate ELISA. The following results were obtained:

Metolachlor Added (ppb)	Mean Recovery (ppb)	S.D. (ppb)	% Recovery
0.5	0.52	0.04	104
1.0	1.08	0.10	108
2.0	2.22	0.15	111
3.0	3.33	0.25	111
4.0	4.19	0.32	105
Average			108

Selectivity: This ELISA recognizes Metolachlor and related acetanilides to varying degrees:

Cross-Reactivity:		
	Metolachlor	100%
	Metolachlor S	100%
	Acetochlor	9%
	Butachlor	9%
	Alachlor	3%
	Metalaxyl	1%

The following compounds demonstrated no reactivity in the ABRAXIS® Metolachlor Plate ELISA at concentrations up to 1000ppb: Aldicarb, Aldicarb Sulfate, Aldicarb Sulfone, Atrazine, Ametryn, Benomyl, Butylate, Captan, Carbaryl, Carbendazim, Carbofuran, Cyanazine, 2,4-D, 1,3-dichloropropene, Dinoseb, MCPA, Metribuzin, Pentachlorophenol, Picloram, Propazine, Simazine, Terbufos, Thiabendazole, and Thiophanate-methyl.

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For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com
Tel.: (215) 357-3911
Fax: (215) 357-5232
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

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ABRAXIS® Metolachlor ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Metolachlor in Contaminated Samples

Product No. 500065

1. General Description

The ABRAXIS® Metolachlor ELISA is an immunoassay for the detection of Metolachlor. This test is suitable for the quantitative and/or qualitative detection of Metolachlor in contaminated samples. Samples requiring regulatory action 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 Metolachlor. 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 the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Metolachlor ELISA kit should to 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 last day of the month as indicated by 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 Metolachlor by specific antibodies. Metolachlor, when present in a sample and a Metolachlor-enzyme conjugate compete for the binding sites of rabbit anti-Metolachlor antibodies in solution. The Metolachlor antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the microtiter 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 Metolachlor present in the sample. The color reaction is stopped after a specified time and the color is evaluated using a microplate ELISA photometer. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® Metolachlor 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. Mistakes in handling the test can also cause errors. Possible sources for such errors can be: 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, exposure to direct or indirect sunlight during the substrate reaction, or extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

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

6. Working Instructions

A. Reagents and Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit).
2. Metolachlor Standards (7): 0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0 ng/mL, 1 mL each
3. Control at 1.5 ± 0.3 ng/mL, 1 mL
4. Antibody Solution (rabbit anti-Metolachlor), 6 mL
5. Metolachlor-HRP Conjugate Solution, 6 mL
6. Sample Diluent, 25 mL. Use to dilute samples.
7. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL

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 (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wavelength 450 nm)
5. Shaker for microtiter plates (optional)
6. Timer
7. Tape or Parafilm

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel, stepping, or electronic repeating pipette is recommended for adding the enzyme conjugate, antibody, substrate (color), and stop solutions in order to equalize the incubation periods across the entire microtiter plate. 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 foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, antibody, conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
5. The stop solution should be handled with care as it contains diluted H_2SO_4.

D. 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; 0.1; 0.25; 0.5; 1.0; 2.0; 5.0 ppb

C: Control: 1.5 +/- 0.3 ppb

Sam1, Sam2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Sam1									
B	Std 0	Std 4	Sam1									
C	Std 1	Std 5	Sam2									
D	Std 1	Std 5	Sam2									
E	Std 2	Std 6	etc.									
F	Std 2	Std 6	etc.									
G	Std 3	C										
H	Std 3	C										

E. Assay Procedure

1. **Add 50 µL of the standard solutions and samples** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. **Add 50 µL of enzyme conjugate solution** to the individual wells successively using a multi-channel pipette, stepping, or electronic repeating pipette.
3. **Add 50 µL of antibody solution** to the individual wells successively a multi-channel pipette, stepping, or electronic repeating 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 contents.
4. **Incubate the strips for 30 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. Please 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 100 µL of substrate (color) solution** to the individual wells successively a multi-channel pipette, stepping, or electronic repeating 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. **Incubate the strips for 20 minutes at room temperature.** Protect the strips from direct sunlight.
7. **Add 50 µL of stop solution** to the individual wells successively a multi-channel pipette, stepping, or electronic repeating pipette 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 the stopping solution.

F. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs 4-Parameter (preferred) or Logit/Log. For 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 Metolachlor concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Metolachlor by interpolation using the standard curve. Samples showing lower concentrations of Metolachlor compared to Standard 1 (0.1 ng/mL) are considered as negative. Samples showing a higher concentration than Standard 5 (5.0 ng/mL) must be diluted further to obtain accurate results.

G. Standard Curve

These values are used for demonstration purposes only; do not use these values for your determinations.