Importance of Trifluralin Determination

Trifluralin is a pre-emergence herbicide used in the control of annual grasses and broadleaf weeds. It is one of the most commonly used herbicides in the United States. Trifluralin is used in both agricultural (cereal grains crops, fruits, vegetables, cotton) and non-agricultural settings (residential lawns, flowers, shrubs).

The U.S. EPA lists Trifluralin as a possible carcinogen. Trifluralin is persistent in the environment, with a half-life in soil of approximately 4 to 7 months. Water sources can be contaminated with Trifluralin through run-off from contaminated ground. Trifluralin is highly toxic to fish and other aquatic organisms. Heating or burning Trifluralin releases toxic and corrosive gasses. Humans may be exposed to Trifluralin through direct physical contact with treated plants or ingestion of contaminated drinking water or foods, including fish from contaminated waters.

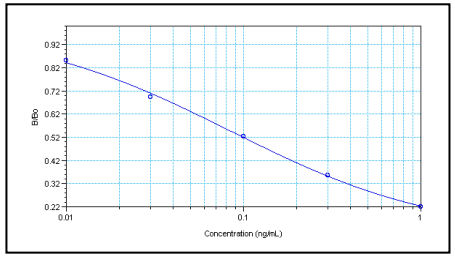
Due to the widespread use of Trifluralin, many countries have established Acceptable Daily Intake (ADI) levels. The United States (US) has established an ADI of 0.0075 mg/kg of body weight. The European Union (EU) has established an ADI of 0.015 mg/kg of body weight. Many countries have also established maximum residue limits (MRLs) on many fruits and vegetables. Due to the widespread contamination of imported shellfish from Vietnam (a country which has banned the use of Trifluralin in aquaculture), Japan has also established an MRL for Trifluralin in fish and shrimp. All imported shrimp must be screened and can contain no more than 0.001 ppm (µg/g).

The ABRAXIS® Trifluralin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of quantitation for Trifluralin (90% B/B_0) is approximately 0.005 ng/mL (0.01 ng/mL in water and 0.165 ng/g in fish or shrimp). The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 0.112 ng/mL (0.224 ng/mL in water and 1.848 ng/g in fish or shrimp). Determinations closer to the middle of the calibration range of the test yield the most accurate results.

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



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

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ABRAXIS® Trifluralin ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Trifluralin in Water, Fish, or Shrimp

Product No. 500601

1. General Description

The ABRAXIS® Trifluralin ELISA is an immunoassay for the detection of Trifluralin in surface water, fish, or shrimp. This test is suitable for the quantitative and/or qualitative detection of Trifluralin in contaminated samples. 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 Trifluralin. 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® Trifluralin 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. The conjugate is supplied in lyophilized form (3 vials). Before each assay, the required volume of lyophilized conjugate must be reconstituted (see Test Preparation section). Reconstitute only the amount needed for the samples to be run, as the reconstituted solution will only remain viable for one week (store frozen).

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Trifluralin by specific antibodies. Trifluralin, when present in a sample and a Trifluralin-enzyme conjugate compete for the binding sites of anti-Trifluralin antibodies in solution. The Trifluralin antibodies are then bound by a second antibody (goat anti-rabbit) 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 Trifluralin 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® Trifluralin 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 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, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

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

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit)
2. Trifluralin Standards (6): 0, 0.01, 0.03, 0.1, 0.3, and 1.0 ng/mL, 1 mL each
3. Trifluralin-HRP Conjugate, 3 vials (lyophilized), must be reconstituted before use, see Test Preparation (Section C)
4. Conjugate Diluent, 12 mL
5. Anti-Trifluralin Antibody Solution, 6 mL
6. Sample Diluent (2), 25 mL
7. Sample Extraction Reagent A, 45 g
8. Sample Extraction Reagent B, 20 g
9. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
10. Substrate (Color) Solution (TMB), 16 mL
11. Stop Solution, 12 mL

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

1. Micro-pipettes with disposable plastic tips (20-200 µL)
2. Multi-channel pipette (50-300 µL), stepper pipette (50-300 µL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Graduated cylinder
5. Container with 500 mL capacity (for 1X diluted Wash Buffer, see Test Preparation, Section C)
6. Tape or Parafilm
7. Timer
8. Paper towels or equivalent absorbent material
9. Glass vials with Teflon-lined caps, 20 mL and 4 mL
10. Methanol, Acetonitrile, Dimethyl Sulfoxide (DMSO)
11. Nitrogen
12. Centrifuge capable of 2800 x g
13. Balance with 0.01 g accuracy
14. Water bath or dry block heater capable of 40-60 °C
15. Microtiter plate reader (wave length 450 nm)

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and samples are necessary. A multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for adding the conjugate, antibody, 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. Allow the microtiter plate, reagents, and samples to reach room temperature before beginning the test.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips must be stored in the foil bag with desiccant and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, antibody, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The conjugate provided is lyophilized (3 vials). Before each assay, calculate the volume of conjugate needed (when reconstituted, each vial will provide enough conjugate for approximately 55 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the conjugate solution will only remain viable for one week (stored frozen). If additional samples are to be analyzed greater than one week after reconstitution, a new vial of conjugate will need to be prepared. To reconstitute, add 3.0 mL of Conjugate Diluent to each vial of conjugate required and vortex thoroughly. If using multiple vials of reconstituted conjugate solution in one assay, reconstitute each vial then combine the reconstituted solutions in a clean amber vial and vortex thoroughly before use.
5. Dilute the ABRAXIS® Wash Buffer (5X) concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
6. The stop solution must be handled with care as it contains diluted H_2SO_4.

D. Sample Preparation

Trifluralin will adsorb to sample containers. As they show a decreased level of adsorption, glass sample bottles and vials are recommended.

Water Samples

Immediately upon collection, water samples should be preserved with methanol at a ratio of 1:1. For example, a 2 mL sample of water added to a glass sample container should have 2 mL of methanol added. Samples should be collected in glass sample containers and tested within 1 week. If samples must be held for longer periods (greater than 1 week), store frozen. The Trifluralin concentration contained in water samples is determined by multiplying the ELISA result by the dilution factor of 2. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed.

Fish or Shrimp Samples

1. Weigh 2 g of homogenized fish or de-shelled shrimp (should have a paste-like consistency) into a 20mL glass vial with a Teflon-lined cap.
2. Add 6 mL of acetonitrile.
3. Add 1 g of Trifluralin Fish/Shrimp Sample Extraction Reagent A. Vortex for 3 minutes.
4. Centrifuge for 10 minutes at 2800 x g. Pipette 1 mL of the supernatant (top layer) into a clean vial. Add 0.4 g of Trifluralin Fish/Shrimp Sample Extraction Reagent B. Vortex for 1 minute. Incubate for 5 minutes at room temperature.
5. Centrifuge for 10 minutes at 2800 x g. Pipette 500 µL of supernatant into a clean vial. Add 75 µL of dimethyl sulfoxide (DMSO). Vortex thoroughly.
6. Evaporate the acetonitrile from the extract at 40-60°C under a gentle stream of nitrogen.
7. Add 100 µL of methanol. Vortex thoroughly to re-dissolve.
8. Add 100 µL of deionized or distilled water. Vortex thoroughly
9. Add 900 µL of Sample Diluent to a clean vial.
10. Add 100 uL of the sample extract (from step 9). Vortex thoroughly. Analyze as sample (Assay Procedure, step 1) as soon as possible after extraction. The Trifluralin concentration contained in fish or shrimp samples is determined by multiplying the ELISA result for samples by the dilution factor of 16.5. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed.

Note: Extracts should be monitored closely, to ensure that the DMSO capture solvent is not also evaporated. A ring of wet, viscous DMSO should be visible at the base of each vial after the acetonitrile has been evaporated. To aid in determining the appropriate appearance of extracts, a comparison vial can be prepared by adding 75 µL of DMSO to a similar clean vial. This will then provide a general approximation of the amount of solvent (DMSO) which should remain after the acetonitrile has evaporated.

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 5: Standards
0; 0.01; 0.03; 0.1; 0.3; 1.0 ppb

Samp1, Samp2, etc.: Samples

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

F. Assay Procedure

1. **Add 50 µL of the standards and 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 antibody solution** to the individual wells successively using a multi-channel, 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 the contents. **Incubate for 30 minutes at room temperature.**
3. **Add 50 µL of reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel, 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 the contents. **Incubate for 30 minutes at room temperature.**
4. 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.
5. **Add 150 µL of substrate (color) solution** to the wells using a multi-channel, 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 for 30 minutes at room temperature.** Protect the strips from direct sunlight.
6. **Add 100 µL of stop solution** to the wells using a multi-channel, stepping, or electronic repeating pipette in the same sequence as for the substrate solution. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stop solution.

G. 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 Trifluralin concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Trifluralin by interpolation using the standard curve; results for water samples are determined by multiplying the concentration determined by the curve by the dilution factor of 2, results for fish or shrimp samples are determined by multiplying the concentration determined by the curve by the dilution factor of 16.5. Samples showing lower concentrations of Trifluralin compared to Standard 1 (0.01 ng/mL) should be reported as containing < 0.02 ng/mL of Trifluralin for water samples or < 0.165 ng/mL for fish or shrimp samples. Samples showing a higher concentration than Standard 5 (1.0 ng/mL) should be reported as containing > 2 ng/mL for water samples or > 16.5 ng/mL for fish or shrimp samples. If a quantitative result is necessary, samples must be diluted further and re-analyzed to obtain accurate results.