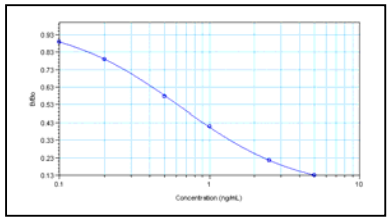
Importance of Benomyl/Carbendazim Determination

Benomyl and its metabolite Carbendazim are fungicides used in the treatment and control of fungal diseases in cereal crops, fruits, vegetables, and ornamental plants, as a seed treatment prior to planting, and in food storage. Although Benomyl was voluntarily removed from the market in 2001 and is no longer in widespread use, Carbendazim continues to be used, frequently in combination with other fungicides. The greatest use of Carbendazim occurs in Europe and Asia. It is among the twelve pesticides most frequently found in European Union (EU) monitoring programs. The maximum residue limits (MRLs) which were initially established in the EU were lowered after the potential harmful effects of Carbendazim were found. Carbendazim is considered to be a potential endocrine disruptor and animal studies have shown in utero exposure to cause severe physical deformities including the lack of formation of eyes and the development of hydrocephalus, or water on the brain. Studies have also shown reproductive effects including impaired testicular development and functioning and infertility. The European Commission has placed Carbendazim on a priority list of chemicals affecting the function of hormones. Carbendazim is also highly toxic to aquatic life. The current EU MRLs for Carbendazim on fresh produce vary according to item, but are in the range of 0.1-0.7 mg/kg. In the United States, Carbendazim is permitted for use only in paints and adhesives, in textiles, and for ornamental trees. It is not approved for use on foods; however, Carbendazim has been found in foods in the US, including baby food in 2000 and imported orange juice in 2012. The monitoring of water sources and food products, including fresh produce and juices, is necessary to ascertain that Carbendazim is not present at levels which present a danger to human health.

The Abraxis Carbendazim ELISA allows the determination of 41 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 Carbendazim (90% B/B_0) is approximately 0.089 ng/mL. The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 0.687 ng/mL. 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%.

Selectivity: This ELISA recognizes Carbendazim and related compounds with varying degrees:

Cross-reactivities:

Carbendazim	100%
Benomyl	70%
Thiabendazole	17%
Thiophanate	0.91%
2-Aminobenzimidazole	0.31%

No cross-reactivity was seen with 2,4-D, Alachlor, Aldicarb, Atrazine, Azinphos, Benzimidazole, Bromophos, Carbofuran, Chlorpyrifos, Metolachlor, Parathion, Simizine, and Terbuthylazine, up to 1,000 ppb (<0.003% cross-reactivity).

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R072512

Benomyl/Carbendazim ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Benomyl/Carbendazim in Water

Product No. 54002B

1. General Description

The Abraxis Benomyl/Carbendazim ELISA is an immunoassay for the detection of Benomyl and Carbendazim in surface water. This test is suitable for the quantitative and/or qualitative detection of Benomyl and Carbendazim 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 Carbendazim. 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 Benomyl/Carbendazim ELISA Kit should to be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until 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 Benomyl and Carbendazim by specific antibodies. Benomyl and Carbendazim, when present in a sample, and a Carbendazim-enzyme conjugate compete for the binding sites of anti-Carbendazim antibodies in solution. The Carbendazim 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 Benomyl/Carbendazim 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 Benomyl/Carbendazim 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 Benomyl/Carbendazim 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.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit).
2. Carbendazim Standards (7): 0, 0.1, 0.2, 0.5, 1.0, 2.5 and 5.0 ng/mL.
3. Carbendazim-HRP Conjugate, 3 vials (lyophilized). Must be reconstituted before use, see Test Preparation (Section C).
4. Conjugate Diluent, 12 mL.
5. Anti-Carbendazim Antibody Solution, 6 mL.
6. Sample Diluent, 25 mL.
7. Wash Solution (5X) Concentrate, 100 mL. Must be diluted before use, see Test Preparation (Section C).
8. Color (Substrate) Solution (TMB), 12 mL.
9. Stop Solution, 6 mL.

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

1. Micro-pipettes with disposable plastic tips (20-200 µL)
2. Multi-channel pipette or stepper pipette (50-250 µL) with disposable plastic tips
3. Deionized or distilled water
4. Graduated cylinder
5. Container with 500 mL capacity (for 1X diluted Wash Solution, see Test Preparation, Section C)
6. Tape or Parafilm
7. Timer
8. Paper towels or equivalent absorbent material
9. Microtiter plate shaker (optional)
10. Microtiter plate washer (optional)
11. 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 or a stepping 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.

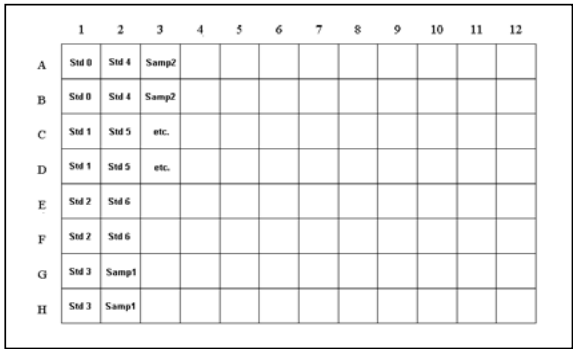
1. Allow the microtiter plate, the reagents, and samples to reach room temperature before beginning the test.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips must be stored in the foil bag with desiccant and zip-locked closed. Store the remaining kit in the refrigerator (4-8°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 wash buffer 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 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; 0.2; 0.5; 1.0; 2.5; 5.0 ppb

Samp1, Samp2, etc.: Samples



	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	Std 6										
F	Std 2	Std 6										
G	Std 3	Samp1										
H	Std 3	Samp1										

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. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of **reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of **antibody 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 strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
4. Incubate the strips for 60 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips **five times** using the 1X washing buffer solution. Use at least a volume of 250 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 stack of paper towels.
6. Add 100 µL of **substrate (color) solution** to the wells using a multi-channel pipette or a stepping 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 30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 50 µL of **stop solution** to the wells using a multi-channel pipette or a stepping 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 Carbendazim concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Carbendazim by interpolation using the standard curve. Samples showing lower concentrations of Carbendazim compared to Standard 1 (0.1 ng/mL) should be reported as containing < 0.1 ng/mL of Carbendazim. Samples showing a higher concentration than Standard 6 (5.0 ng/mL) must be diluted further to obtain accurate results.