

# Benomyl/Carbendazim Plate Kit

PN 54002B

Instructional Booklet  
Read Completely Before Use.

## Intended Use

The Abraxis LLC Carbendazim/MBC Plate Kit is an immunological laboratory test for the quantitation of carbendazim residues in water in the range of 0.2 to 5.0 ng/mL (parts per billion or ppb). Samples containing higher concentrations can be measured by pre-dilution of the sample.

## Test Principles

The Abraxis LLC Carbendazim/MBC Plate Kit uses polyclonal antibodies that bind both benomyl/carbendazim and a carbendazim-enzyme conjugate. Carbendazim in the sample competes with carbendazim-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind benomyl/carbendazim compounds, are immobilized to the inside of the test wells. In the assay procedure you will:

- Add a sample containing benomyl/carbendazim to a test well, followed by carbendazim-enzyme conjugate, and anti-carbendazim antibody. The conjugate competes with any benomyl/carbendazim in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 60 minutes.
- Add clear substrate solution to each well. In the presence of bound carbendazim-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of carbendazim-enzyme conjugate molecules, a sample containing a low concentration of benomyl/carbendazim allows the antibody to bind many carbendazim -enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of carbendazim allows fewer carbendazim -enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

**NOTE:** Color is inversely proportional to benomyl/carbendazim concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

## Specificity

The Abraxis LLC Benomyl/Carbendazim Plate Kit is specific for benomyl/carbendazim and closely related compounds. The following table shows the concentration required for 50% Bo and the % cross-reactivity versus carbendazim (used in calibrators). Concentrations are in parts per billion (ppb).

<u>Compound</u>	<u>50% Bo conc.</u>	<u>% Cross Reactivity</u>
Carbendazim	0.091	100
Benomyl	0.13	70
Thiabendazole	0.54	17
Thiophanate	10	0.91
2-aminobenzimidazole	29	0.31

The following list shows the compounds tested and found non-reactive at concentrations of 1,000 ppb (<0.003% cross-reactivity):

Alachlor	Aldicarb	Atrazine
Azinphos	Bromophos	Terbutylazine
Carbofuran	Chlorpyrifos	2,4-D
Metolachlor	Parathion	Simazine
Benzimidazole		

### Precautions

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Due to Stability issues, reconstituted Enzyme Conjugate **must be used immediately after reconstitution**, unused portions should be discarded.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents from kits with different lot numbers.
- Use approved methodologies to confirm any positive results.

### Materials Provided in the Abraxis LLC Benomyl/Carbendazim Plate Kit

- 1 plate each containing 12 strips of 8 wells coated with rabbit anti-rabbit antibodies
- 1 vial each of rabbit anti-benomyl/carbendazim antibodies
- 1 vial each of carbendazim calibrators corresponding to 0, 0.2, 0.5, 1.0, 2.5 and 5.0 ppb.
- 4 vials of lyophilized Carbendazim-HRP Enzyme Conjugate (purple dot). Reconstitute 1 vial with 2 mL of conjugate diluent (provided) and vortex or swirl for 2 minutes to dissolve. **Reconstituted HRP conjugate must be used immediately after reconstitution** (1 vial of enzyme conjugate is enough for approximately 40 wells)
- 1 vial of Conjugate Diluent
- 1 vial of Substrate (Color Solution)

1 vial of Stop Solution

1 bottle of Wash Buffer (5X concentrated). Dilute the wash buffer at a ratio of 1:5. If using the entire bottle (100 mL) then add to 400 mL of deionized or distilled water.

**You also need these items:**

- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Wash bottle
- Deionized or distilled water
- Orbital shaker (optional)
- Pipet with disposable tips capable of delivering 100  $\mu$ L

**Assay Procedure**

1. Bring all kit reagents and samples to be analyzed to room temperature.
2. Remove the required number of antibody coated strips from the zip lock bag. Be sure to re-seal the bag with the dessicant to limit exposure of the strips to moisture.
3. Pipet **50  $\mu$ L of calibrators and samples** into the appropriate wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
4. Add **50  $\mu$ L of Enzyme Conjugate** to each well.
5. Add **50  $\mu$ L of Antibody Solution** to each well.
6. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotator for continuous mixing during incubation.
7. Incubate for **60 minutes**.
8. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with diluted Wash Buffer (1X), then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
9. Add **100  $\mu$ L of Substrate** to each well.
10. Cover the wells and incubate for **30 minutes**.
11. Add **50  $\mu$ L of Stop Solution** to each well in the same order of addition as the Substrate.
12. Read the plate on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
13. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4 parameter curve fit. If manual data reduction is required, proceed with next section.

## Calculate Results

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

$$\%Bo = (\text{average OD of calibrator or sample} \times 100) \div \text{average OD of negative control}$$

2. Graph the %Bo of each calibrator on the Y (linear) axis against its carbendazim concentration on the X (log) axis using semi-log graph paper. Draw the best fit line through the calibrator points.
3. Determine the benomyl/carbendazim concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.

Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

## Sample Calculations

Well Contents	OD	Average OD $\pm$ SD**	%RSD	%Bo
Negative Control	1.184 1.234	1.209 $\pm$ 0.0035	2.9	100.0
0.2 ppb Calibrator	0.991 0.987	0.989 $\pm$ 0.003	0.3	81.8
0.5 ppb Calibrator	0.810 0.809	0.810 $\pm$ 0.001	0.1	67.0
1.0 ppb Calibrator	0.600 0.619	0.610 $\pm$ 0.013	2.2	50.5
2.5 ppb Calibrator	0.359 0.358	0.359 $\pm$ 0.001	0.2	29.7
5.0 ppb Calibrator	0.215 0.218	0.217 $\pm$ 0.002	1.0	18.0

Alternatively, Abraxis can supply a 4-parameter spreadsheet template which can be used for data reduction. Please contact Abraxis for further details.

## Technical Assistance

For questions regarding this kit or for additional information about Abraxis LLC products, call (215) 357-3911.

## Safety

To receive complete safety information on this product, contact Abraxis LLC and request Material Safety Data Sheets.

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