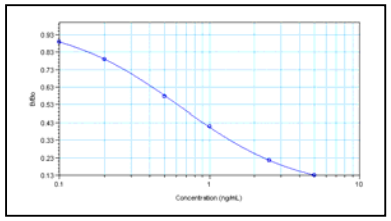
Importance of Benomyl/Carbendazim Determination

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).

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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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.

Life Technologies™
Your Molecular & Cell Technology Partner

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.

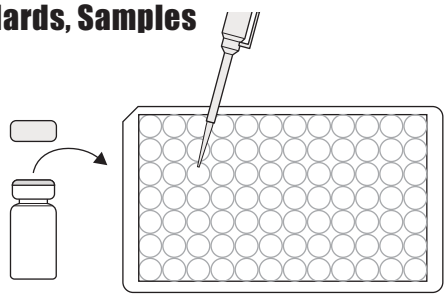
F. Evaluation

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.

Benomyl/Carbendazim Plate, Detailed ELISA Procedure

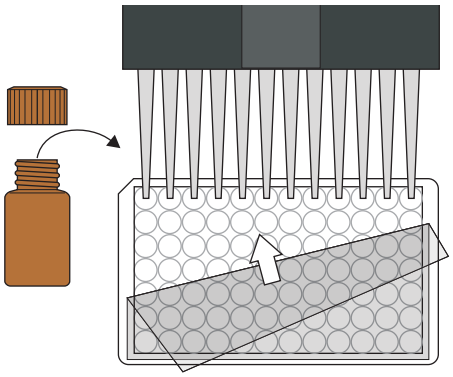
1. Addition of Standards, Samples

Add 50 uL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



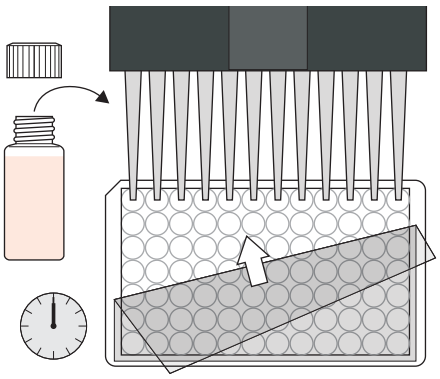
2. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



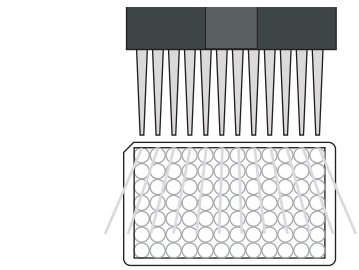
3. Addition of Antibody Solution

Add 50 uL of the antibody solution to the individual wells successively using a multi- channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 60 min at room temperature.

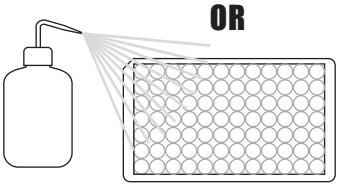


4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips five times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 250 uL 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.

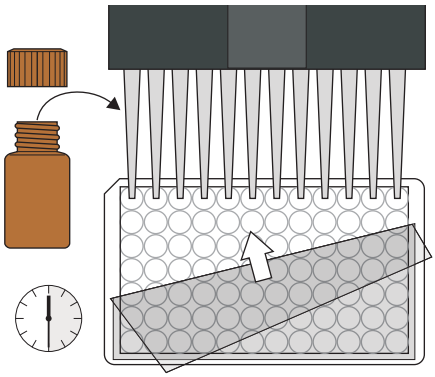


OR



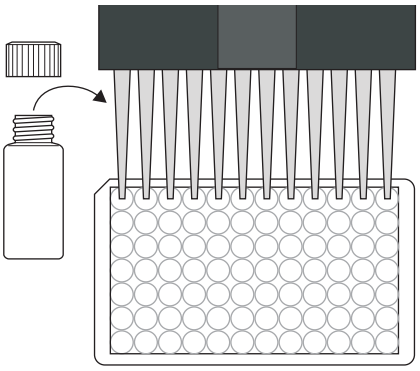
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color 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 rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



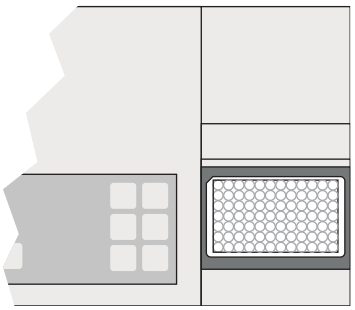
6. Addition of Stopping Solution

Add 50 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.



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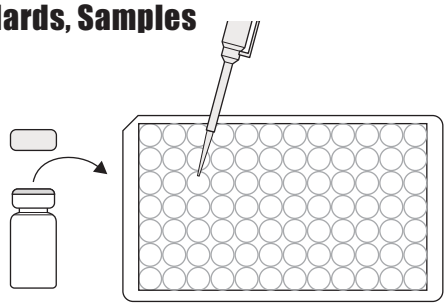
Benomyl/Carbendazim Plate, Concise ELISA Procedure

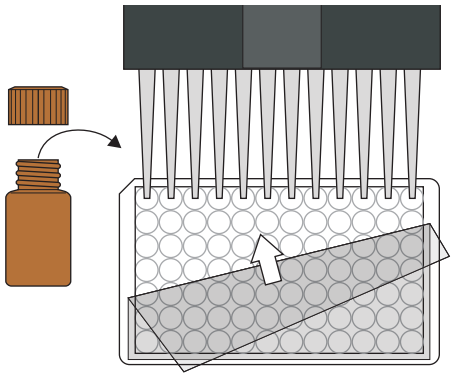
1. Addition of Standards, Samples

Add 50 uL of standard solutions, control or samples.



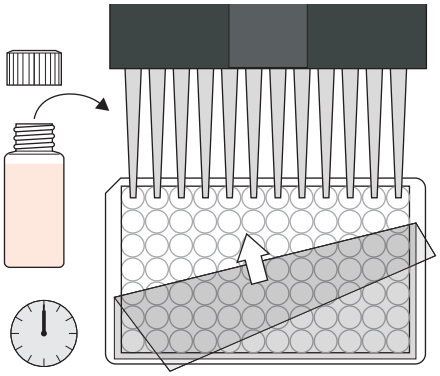
2. Addition of Enyzme Conjugate

Add 50 uL of enzyme conjugate.



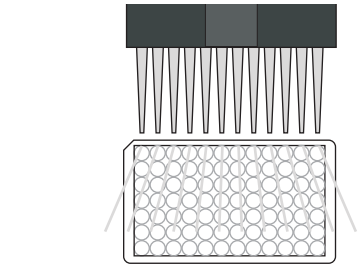
3. Addition of Antibody Solution

Add 50 uL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 60 minutes at room temperature.

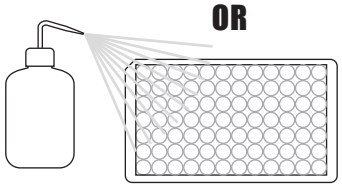


4. Washing of Plates

Wash the plates five times with 250 uL of diluted 1X washing buffer.

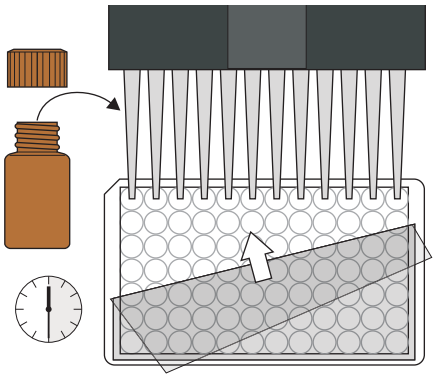


OR



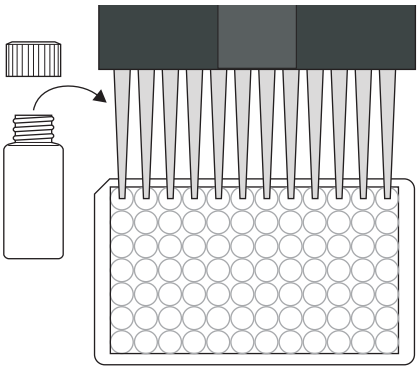
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 30 minutes at room temperature and away from direct sunlight.



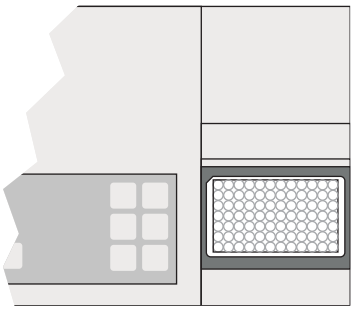
6. Addition of Stopping Solution

Add 50 uL of stop solution.



7. Measurement of Color

Measure color at 450 nm. Calculate results.



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Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: Carbendazim/Benomyl ELISA Plate Kit
Product Code: 54002B
1.2 Identified Use: Determination of Carbendazim/Benomyl in samples. **Restrictions on Use:** For research use only.
1.3 Company: Abraxis, Inc., 124 Railroad Drive, Warminster, PA 18974 USA, info@abraxiskits.com +1(215) 357-3911, FAX +1(215) 357-5232
1.4 Emergency Telephone Number: +1(215) 357-3911

Section 2: Hazard(s) Identification

2.1 Classification of the mixture: Not a hazardous mixture.
2.2 GHS Label elements, including precautionary statements: Not applicable.
2.3 Hazards not otherwise classified (HNOC) or not covered by GHS: None known.
2.4 Unknown acute toxicity: None known.

Section 3: Composition / Information on Ingredients

3.2 Mixtures: *Contains no hazardous ingredients at levels requiring disclosure by the OSHA Hazard Communication Standard (29 CFR 1910.1200)*, however it contains minor amounts of materials considered hazardous. We recommend handling all substances with caution.

Section 4: First Aid Measures

4.1 Description of first aid measures: Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.
If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact: Wash off with soap and plenty of water. Consult a physician.
In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
4.2 Most important symptoms and effects, both acute and delayed: No data available
4.3 Indication of any immediate medical attention and special treatment needed: No data available. Treat symptomatically.

Section 5: Fire-fighting Measures

5.1 Suitable extinguishing media: Use an extinguishing agent suitable for the surrounding fire.
5.2 Special hazards arising from the substance or mixture: None known
5.3 Advice for firefighters: Wear self-contained breathing apparatus for fire-fighting if necessary.
5.4 Further information: No data available

Section 6: Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures: Use personal protective equipment (see section 8). Avoid dust formation. Avoid breathing vapors, mist, dust, or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
6.2 Environmental precautions: Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.
6.3 Methods and materials for containment and cleaning up: Solids (if applicable): Pick up and arrange disposal without creating dust. Sweep up and shovel. Liquids (if applicable): Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Keep in suitable, closed containers for disposal.
6.4 Reference to other sections: For information on safe handling see section 7.
For information on personal protection see section 8.
For information on disposal see section 13.

Section 7: Handling and Storage

7.1 Precautions for safe handling: See section 2. Avoid inhalation of vapors and contact with skin and eyes. Wear appropriate personal protective equipment. Do not eat, drink, or smoke in work area.
7.2 Precautions for safe storage: Keep container(s) tightly closed in a dry, well-ventilated place. Protect from physical damage. See label or product insert for appropriate storage temperature and additional specific information.

7.3 Specific end use(s): No data available

Section 8: Exposure Controls / Personal Protection

8.1 Control parameters: Not applicable.
8.2 Exposure controls:
Appropriate engineering controls: Provide adequate ventilation. Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday. Keep away from food and beverages.
Personal protective equipment: The usual precautionary measures, including eye/face/skin protection, should be taken when handling any chemical. Avoid contact with eyes, skin, and clothing.
Eye protection: As with handling of any chemical, wear approved safety goggles.
Skin protection: Handle with gloves. No specific information regarding glove material or thickness is available, but gloves must be impermeable and resistant to the substance being handled. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Respiratory protection: As with any chemical, where excessive vapor, mist, or dust may result, use a chemical fume hood or approved respiratory protection equipment.
Body protection: Lightweight, protective clothing.

Section 9: Physical and Chemical Properties

9.1 Information on basic physical and chemical properties
Appearance: Multiple
Odor: Characteristic
Odor Threshold: No data available
pH: Multiple
Melting point/freezing point: No data available
Initial boiling point and boiling range: No data available
Flash point: Not applicable
Evaporation rate: No data available
Flammability (solid, gas): No data available
Upper/lower flammability or explosive limits No data available
Vapor pressure: No data available
Vapor density: No data available
Relative density: No data available
Water solubility: Various
Partition coefficient: n-octanol/water: No data available
Auto-ignition temperature: Not applicable
Decomposition temperature: No data available
Viscosity: No data available
Explosive properties: No data available
Oxidizing properties: No data available
9.2 Other information: No data available

Section 10: Stability and Reactivity

10.1 Reactivity: No data available
10.2 Chemical stability: Stable under recommended storage conditions.
10.3 Possibility of hazardous reactions: No data available
10.4 Conditions to avoid: No data available
10.5 Incompatible materials: No data available
10.6 Hazardous decomposition products: No data available. In the event of fire: see section 5.

Section 11: Toxicological Information

11.1 Information on toxicological effects
Acute toxicity: Not available. To the best of our knowledge, the chemical, physical, and toxicological properties of this product have not been thoroughly investigated.
Inhalation: No data available **Ingestion:** No data available **Skin contact:** Irritant to skin and mucous membranes.
Eye contact: May cause eye irritation in susceptible persons. **Respiratory or skin sensitization:** No data available
Aspiration hazard: No data available **Mutagenicity:** No data available

Carcinogenicity
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.
Teratogenicity: No data available
Reproductive/fertility toxicity: No data available
Specific target organ toxicity, single exposure: No data available
Specific target organ toxicity, repeated exposure: No data available

Section 12: Ecological Information

12.1 Toxicity: No data available
12.2 Persistence and degradability: No data available
12.3 Bioaccumulative potential: No data available
12.4 Mobility in soil: No data available
12.5 Results of PBT and vPvB assessment: No data available
12.6 Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13: Disposal Considerations

13.1 Waste treatment methods
Product: All waste must be handled and disposed according to local, state, and federal regulations. Avoid disposing large volumes in sewer.
Contaminated packaging: All waste must be handled and disposed according to local, state, and federal regulations.
Refer to sections 7 and 8 for safe handling guidance.

Section 14: Transport Information

UN Number: Not regulated
UN Proper shipping name: Not classified as dangerous in the meaning of transport regulations.
Transport hazard class(es): No data available
Packing group: No data available
Environmental hazard: No data available
Bulk transport: No data available
Special considerations: No data available

Section 15: Regulatory Information

To the best of our knowledge, this product contains no substances which, at their given concentrations, are considered hazardous by other regulatory agencies. Refer to section 3.

Section 16: Other information

This information is based on our present knowledge. While Abraxis , Inc. believes that the data contained herein are factual and the opinions expressed represent a best effort to present accurate information, the data are not to be taken as a warranty or representation for which Abraxis , Inc. assumes legal responsibility. The information shall not be taken as being all-inclusive and is to be used only as a guide. The data are offered solely for the user's consideration, investigation, and verification. These suggestions should not be confused with either state, municipal, or insurance requirements, or with national safety codes and constitute no warranty. Any use of these data and information must be determined by the user to be in accordance with applicable federal, state, and local regulations.

All materials and mixtures may present unknown hazards and should be used with caution. Since Abraxis , Inc. cannot control the methods, volumes, or conditions of use of this product, Abraxis , Inc. shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material. This product is sold for research use only. It is not for any human or animal therapeutic or clinical diagnostic use.

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Changes from previous version: Abraxis, LLC changed to Abraxis, Inc.