Importance of Microcystins/Nodularins Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 80 variants of Microcystins have been isolated. The most common variant is Microcystin-LR. Other common Microcystin variants include LA, YR, RR, LF, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis, Anabaena, Oscillatoria, Nostoc, Anabaenopsis,* and terrestrial *Hapalosiphon.* Nodularins are produced by the genus *Nodularia* and are found in marine and brackish water.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has lead to death. Human and animal exposure to these toxins occurs most frequently through ingestion of water, through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore may act as tumor promoters.

To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water. In June 2015, USEPA issued Health Advisories (HAs) for Microcystins in drinking water of 0.3 ppb for children pre-school age and younger (less than six years of age) and 1.6 ppb for school age children through adults.

Performance Data

Test sensitivity: The detection limit, based on Microcystins-LR, (90% B/B_0) is approximately 0.09 ppb (µg/L). The middle of the test (50% B/B_0) is approximately 0.719 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Precision:

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppb)	0.518	1.590	3.134
%CV (within assay)	11.1	8.0	11.3
%CV (between assay)	6.5	5.4	7.6

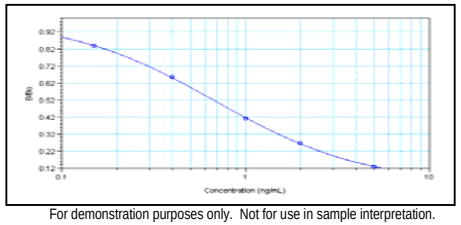
Recoveries:

Level	Mean	%CV	%Recovery
0.25	0.247	14.1	98.8
0.50	0.527	9.1	105.4
1.50	1.560	9.9	104.0
3.00	3.179	9.2	106.0

Specificity: Cross-reactivity of the Abraxis Microcystins Tube Kit for various congeners:

Microcystin-LR	100%
Nodularins	104%
Microcystin-RR	75%
Microcystin-LA	64%
Microcystin-LW	64%
Microcystin-YR	58%
Microcystin-LF	42%

Standard Curve:



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

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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ABRAXIS

Microcystins Tube Kit

Enzyme-Linked Immunosorbent Assay for the Determination of Microcystins and Nodularins in Water Samples

Product No. 520012A

1. General Description

The Abraxis Microcystins Tube Kit is an immunoassay for the quantitative and sensitive screening of Microcystins and Nodularins in water samples. This test is suitable for the quantitative and/or qualitative screening of Microcystins and Nodularins in drinking and recreational water samples (please refer to Sample Collection and Handling, section C). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Microcystins. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The Microcystins Tube Kit should 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. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Microcystins, Nodularins, and their congeners by specific antibodies (see Performance Data). The Microcystins, when present in a sample, and a Microcystins-enzyme conjugate compete for the binding sites of rabbit anti-Microcystins antibodies in solution. The Microcystins antibodies are then bound by a second antibody (anti-rabbit) immobilized on the test tube. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Microcystins Tube Kit, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

Samples containing methanol must be diluted to a concentration < 40% methanol to avoid matrix effects.

When performing the test exposure to direct sunlight must be avoided.

Seawater samples up to 37 parts per thousand were tested and no matrix effects were detected. Average recovery of spiked seawater samples was 104.6%.

No matrix effects have been observed with samples that have been treated with sodium thiosulfate at concentrations ≤ 1 mg/mL or ascorbic acid at concentrations ≤ 1 mg/mL.

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

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. 40 test tubes coated with a secondary antibody (anti-rabbit), in a resealable aluminum pouch
2. Microcystins-HRP Enzyme Conjugate, 25 mL
3. Anti-Microcystins Antibody Solution, 25 mL
4. Standards (6): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb, 4 mL each
5. Control at 0.75 ± 0.19 ppb, 4 mL
6. HA Control at 0.30 ± 0.07 ppb, 4 mL
7. Wash Solution (100X) Concentrate, 25 mL, must be diluted before use, see Test Preparation (Section D)
8. Substrate (Color) Solution (TMB), 25 mL
9. Stop Solution, 25 mL (handle with care)

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

1. Micro-pipettes with disposable plastic tips (500 µL)
2. Stepper pipette with disposable plastic tips (12.5 mL-50 mL)
3. Test tube rack capable of holding 12 mm test tubes *securely*
4. Photometer capable of reading 12 mm tubes at 450 nm
5. Vortex mixer
6. Deionized or distilled water
7. Paper towels or equivalent absorbent material
8. Timer

C. Sample Collection and Handling

Collect water samples in glass or PETG containers and test within 24 hours. Drinking water samples should be treated with sodium thiosulfate (up to 1 mg/mL) or ascorbic acid (up to 1 mg/mL) immediately after collection to remove residual chlorine. If samples must be held for longer periods (up to 5 days), samples should be stored refrigerated. For storage periods greater than 5 days, samples should be stored frozen.

If total Microcystins concentration (free and cell bound) is required, an appropriate cell lysing procedure (three freeze and thaw cycles are recommended) must be performed prior to analysis. *Note: The use of sonication in cell lysing can negatively affect toxin concentrations, producing falsely low sample results.*

Samples may be filtered prior to analysis using glass fiber filters (Environmental Express 1.2 µm syringe filters, part number SF012G, are recommended). If determining total Microcystins concentration, samples should be lysed prior to filtration to prevent the removal of cell-bound Microcystins, which would cause inaccurate (falsely low) results. *Note: The use of alternate filter types (non-glass fiber filters) may produce falsely low sample results, as Microcystins may bind to the filter material, removing it from the sample.*

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods across the entire assay, a stepping pipette is recommended for adding the enzyme conjugate, antibody, diluted wash, substrate, and stop solutions. Please only use the coated tubes, reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the coated test tubes, reagents, and samples to ambient temperature before use.
2. After removing the required number of coated test tubes from the aluminum pouch, seal the remaining test tubes in the pouch with the desiccant.
3. The conjugate, standard solutions, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. After adjusting to ambient temperature *(Note: Some salts may settle out upon refrigeration, but will redissolve when adjusted to ambient temperature and mixed thoroughly.)*, dilute the Wash Solution (100X) Concentrate at a ratio of 1:100 with deionized or distilled water (i.e., 1 mL of Wash Solution Concentrate added to 100 mL of deionized or distilled water) and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.
6. After analysis, store the remaining kit components in the refrigerator (4-8°C).

E. Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while avoiding contact between the reagents and the pipette tip. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contamination and carryover of reagents by using clean pipette tips for each standard/sample addition and by avoiding contact between reagent droplets on the tubes and pipette tips.

Avoid foam formation during vortexing.

F. Assay Procedure

1. Remove the required number of coated test tubes from the re-sealable aluminum pouch. Place the tubes in a rack capable of holding 12 mm test tubes *securely* and label appropriately. Analysis in duplicate is recommended.
2. Add 500 µL of **enzyme conjugate solution** to the coated tubes successively using a stepping pipette.
3. Add 500 µL of the **standard solutions**, **controls**, or **samples** into the appropriate coated tubes.
4. Add 500 µL of **antibody solution** to the coated tubes successively using a stepping pipette. Carefully vortex the tubes at a low speed for 1 to 2 seconds or swirl the tubes rapidly allowing the contents to mix and being careful not to spill or splash the contents.
5. Incubate the tubes for 20 minutes at room temperature.
6. Decant the contents of the tubes by vigorously shaking into a sink. Blot the inverted tubes on absorbent paper towels. Flood the tubes with 5 mL of **diluted washing buffer solution**, decant by shaking vigorously into a sink, and blot the inverted tubes on absorbent paper towels. Repeat four times for a total of **five washes**.
7. Add 500 µL of **substrate (color) solution** to the tubes. Carefully vortex the tubes at a low speed for 1 to 2 seconds or swirl the tubes rapidly, being careful not to spill or splash the contents.
8. Incubate the tubes for 20 minutes at room temperature. Protect the tubes from direct or indirect sunlight.
9. Add 500 µL of **stop solution** to the tubes in the same sequence as for the substrate solution.
10. Read the absorbance at 450 nm using a test tube photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter. Results can be determined using a spreadsheet macro (4-Parameter) available from Abraxis upon request. 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 Microcystins concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control and samples will then yield levels in ppb (ng/mL) of Microcystins by interpolation using the standard curve.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Microcystins than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of Microcystins. Samples showing a higher concentration than standard 5 (5.0 ppb) should be reported as containing > 5.0 ppb Microcystins or must be diluted to obtain accurate results. Samples maybe diluted in deionized or distilled water if necessary.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of Microcystins greater than the concentration of that standard. Samples with higher absorbances than a standard will have concentrations of Microcystins less than that standard.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

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MICROCYSTIN DETAILED FLOWCHART

1.

Add 500 µL of Microcystin Enzyme Conjugate down the inside wall of each tube by using the technique described in Box 2.

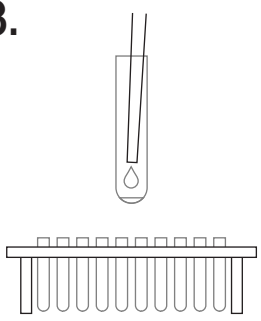
2.

Label test tubes for Standards (Calibrators), Control, and Samples.

Tube #	Content
1, 2	Diluent/Zero Standard 0 ppb
3, 4	Standard 1, 0.15 ppb
5, 6	Standard 2, 0.40 ppb
7, 8	Standard 3, 1.0 ppb
9, 10	Standard 4. 2.0 ppb
11,12	Standard 5, 5.0 ppb
13	Control, 0.75 ppb
14	Sample 1
15	Sample 2
16	Sample 3

Add 500 µL of either Standards, Control or Samples down the inside wall of each test tube by aiming the pipet tip 1/4" to 1/2" below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

3.



Add 500 µL of the Microcystin Antibody Solution to the bottom of each tube by inserting the pipette tip all the way into the bottom of the tube without touching the side of the tubes. *Vortex or swirl* for 5 to 10 seconds.

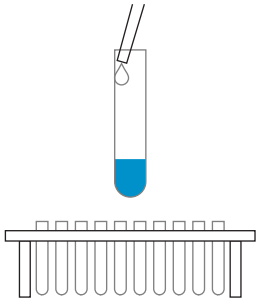
4.

React 20 minutes at room temperature (15 °- 30°C). After incubating, invert and shake over sink. Blot in absorbent paper.

5.

Add 5 mL of Washing Solution to each tube (alternatively flood the tubes completely with wash solution then invert to empty tubes). Vigorously shake and invert tubes over a sink and pour out the tube contents: keep inverted and blot the test tube rims on several layers of paper toweling.
Repeat this step 4 times.

6.

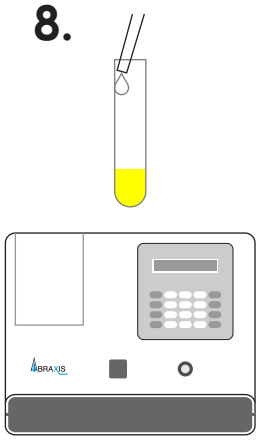


Add 500 µL of Color Reagent down the inside wall of each tube by using the technique described in Box 2. Vortex or swirl.

7.

React for 20 minutes at room Temperature (15°- 30° C). During this period, add 1 mL of Washing Solution into a clean tube for use as an instrument blank in Step 8.

8.



Add 500 µL of Stopping Solution down the inside wall of each tube by using the technique previously Described. *Read* results at 450 nm within 15 minutes after adding the Stopping Solution. *Multiply* results of samples by the appropriate dilution factor (if any).

[**Safety Caution:** Stopping Solution contains diluted sulfuric acid.]

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MICROCYSTIN CONCISE FLOWCHART

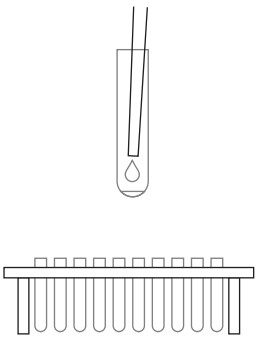
1.

Add 500 µL of Microcystin Enzyme Conjugate to each test tube.

2.

Add 500 µL of either Standards, Control or Samples to the bottom of each test tube.

3.



Add 500 uL of Microcystin Antibody Solution to each test tube.

Vortex or swirl.

4.

Incubate for 20 minutes.
Invert and blot.

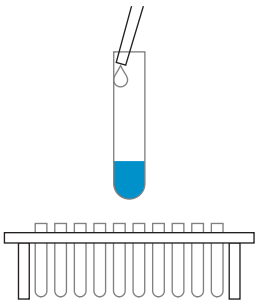
5.

Add 5 mL of Washing Solution (alternatively flood the tubes).

Invert the tubes and blot.

Repeat this step 4 times.

6.



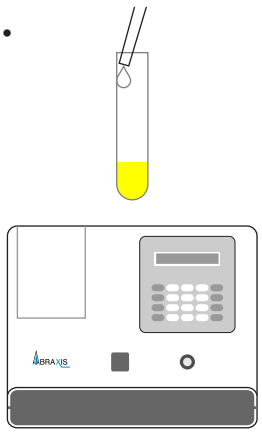
Add 500 µL of Color Reagent down the inside wall of each test tube.

7.

Incubate for 20 minutes.

Prepare blank.

8.



Add 500 µL of Stopping Solution to each test tube.

Read OD 450

Microcystin Tube Kit Part # 520012A

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ABRAXIS

Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: Microcystins Coated Tube Kit
Product Code: 520012a
1.2 Identified Use: Determination of Microcystins 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.

Date this SDS was prepared: 5/24/2016
Version: 2
Changes from previous version: Abraxis, LLC changed to Abraxis, Inc.