Importance of β-*N*-methylamino-L-alanine (BMAA) Determination

β-*N*-methylamino-L-alanine (BMAA) is a non-protein amino acid produced by several types of cyanobacteria found in freshwaters, marine waters, and soil. When ingested, BMAA damages and ultimately destroys motor neurons in the spinal cord, causing the same type of damage seen in patients with ALS, and causes neurofibrillary tangles in the spinal cord and brain, similar to those seen in Alzheimer's disease. Those suffering from this BMAA-induced damage are classified as suffering from amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC). The symptoms of ALS/PDC include the varying degrees of the muscular paralysis of ALS, the muscular rigidity of Parkinson's, and/or the dementia of Alzheimer's and ultimately result in death.

Humans may be exposed to BMAA through the ingestion of contaminated drinking water or foods. Drinking water may become contaminated with BMAA through the proliferation of BMAA-producing cyanobacteria in drinking water sources such as lakes and reservoirs. It is unknown whether the various methods of water treatment are able to remove BMAA from the drinking water supply. Exposure also occurs thorough ingestion of foods such as cycad seeds, on whose plant roots BMAA-producing cyanobacteria are known to live and thereby contaminate the plant and its seeds, and through the ingestion of fish or other animals which have consumed toxin containing plants or cyanobacteria. An example of this biomagnification was seen in Guam in the 1950s, when fruit bats, which consume cycad seeds, were consumed by the native Chamorro people causing a dramatic increase in cases of ALS/PDC, known on Guam as "lytico-bodig."

The BMAA ELISA allows for the analysis of 42 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in approximately 2 hours.

Performance Data

Test sensitivity: The limit of quantitation for BMAA (95% B/B_0) is approximately 4 ng/mL. The middle of the test (50% B/B_0) is approximately 100 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Sample concentration may be performed for samples requiring a lower limit of detection (technical bulletin available from Abraxis by request).

Sample extraction and clean-up are necessary for biological samples (please refer to Z. Spacil, et al., *Analyst* 2010, **135**, 127-132)

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

Specificity: Cross-reactivity of the Abraxis BMAA ELISA for related compounds:

β-*N*-methylamino-L-alanine (BMAA)	100%
L-Cysteine hydrochloride	0.2%
L-Glutamic acid	0.2%
L-Aspartic acid	0.2%
γ-Aminobutyric acid	0.02%
DL-2,4-Diaminobutyric acid dihydrochloride	0.01%

No cross-reactivity was seen with Glycine, L-Isoleucine, L-Lysine monohydrochloride, L-Histidine monohydrochloride monohydrate, L-Tryptophan, L-Alanine, L-Tyrosine, L-Valine, L-Cystine, L-Asparagaine, L-Phenylalanine, L-Threonine, L-Proline, L-Arginine monhydrochloride, L-Glutamine, L-Methionine, trans-4-hydroxy-L-proline, L-Serine, and L-Leucine up to 1 µg/mL.

No cross-reactivity was seen with Microcystin-LR and Cylindrospermopsin up to 0.1 µg/mL.

For ordering or technical assistance contact:

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ABRAXIS

β-*N*-methylamino-L-alanine (BMAA) ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of β-*N*-methylamino-L-alanine (BMAA) in Water Samples

Patent Number 8,394, 596

Product No. 520040

1. General Description

The Abraxis β-*N*-methylamino-L-alanine (BMAA) ELISA is a patented immunoassay for the quantitative and/or qualitative screening of BMAA in water samples. This assay is not intended for intended for Clinical Diagnostics.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of BMAA. 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 BMAA ELISA 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. The standards are supplied in lyophilized form and must be reconstituted before use (see Test Preparation, Section D). Reconstituted standards may be used for up to one month (store frozen).

4. Test Principle

The test is a direct competitive ELISA based on the recognition of BMAA by specific antibodies. BMAA, when present in a sample, and a BMAA-HRP analogue compete for the binding sites of the rabbit anti-BMAA antibodies in solution. The BMAA antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. 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 BMAA 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 BMAA ELISA, 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 samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the BMAA Kit: manganese sulfate and aluminum oxide up to 10,000 ppm; calcium chloride and sodium thiosulfate up to 1000 ppm; potassium phosphate, ferrous sulfate, and zinc sulfate up to 100 ppm; copper chloride, calcium sulfate, magnesium sulfate, sodium fluoride, and sodium nitrate up to 10 ppm; sodium chloride up to 1 ppm; humic acid and magnesium chloride up to 0.1 ppm; methanol up to 1%; and seawater up to 10%.

Mistakes in handling the test 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, and extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

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

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A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Lyophilized BMAA Calibrators/Standards (6): 0, 5, 25, 100, 250, 500 ng/mL (ppb), must be reconstituted before use, see Test Preparation (Section D)
3. Antibody Solution (rabbit anti-BMAA), 6 mL
4. BMAA-HRP Conjugate Solution, 6 mL
5. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
6. Sample Diluent, 25 mL
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL (handle with care)

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

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Deionized or distilled water
5. Paper towels or equivalent absorbent material
6. Timer
7. Tape or parafilm

C. Sample Collection

Collect water samples in polypropylene (PP) or high density polyethylene (HDPE) containers. **Do not use glass containers, as analyte will be lost due to adsorption.**

C. Test Preparation

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

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The conjugate, antibody, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The standards are provided in lyophilized form. To reconstitute, add 1 mL of deionized or distilled water to each standard vial. Vortex thoroughly. Reconstituted standards may be used for up to one month (store frozen).
5. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
6. The stop solution must 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 5: Standards
(0; 5; 25; 100; 250; 500 ppb)

Samp1, Samp2, etc.: Samples

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

E. Assay Procedure

1. Add 100 µL of the **calibrator/standard solutions or 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 **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 90 minutes at room temperature.
5. Remove the covering and decant the contents of the wells into a sink. Wash the strips **four times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
6. Add 150 µL of **substrate (color) solution** to the wells. 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 the strips for 30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of **stop solution** to the wells 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 stopping solution.

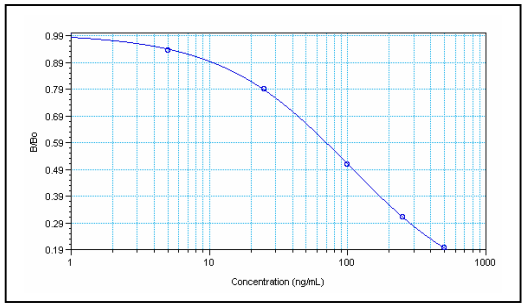
F. Evaluation

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

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of BMAA than standard 1 (5 ppb) should be reported as containing < 5 ppb of BMAA. Samples showing a higher concentration than standard 5 (500 ppb) must be diluted further with the provided sample diluent and re-analyzed.

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

G. Standard Curve



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

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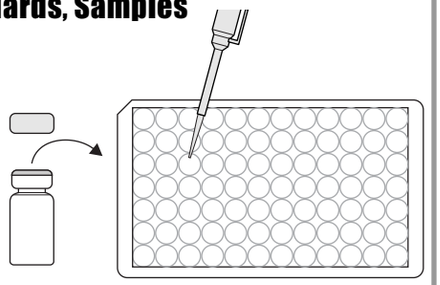
BMAA Plate, Detailed ELISA Procedure

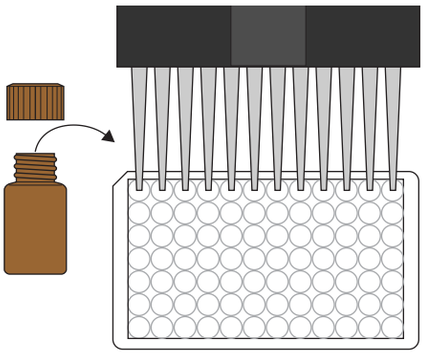
1. Addition of Standards, Samples

Add 100 uL of the standard solutions or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



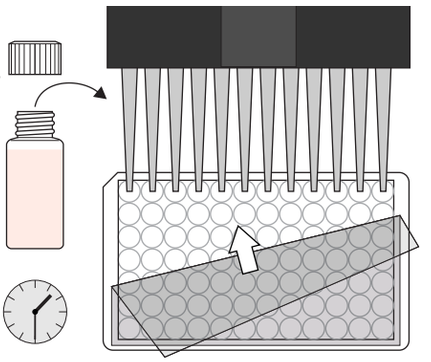
2. Addition of Enyzme Conjugate

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



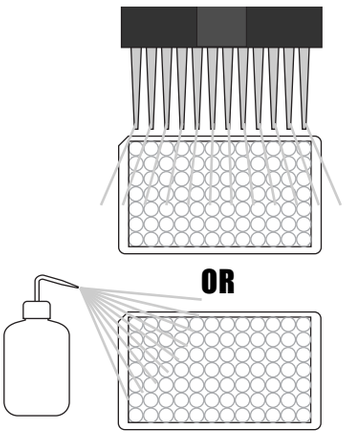
3. Addition of Antibody Solution

Add 50 uL of the BMAA 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 circular motion on the benchtop. Be careful not to spill the contents. Incubate the strips for 90 minutes at room temperature.



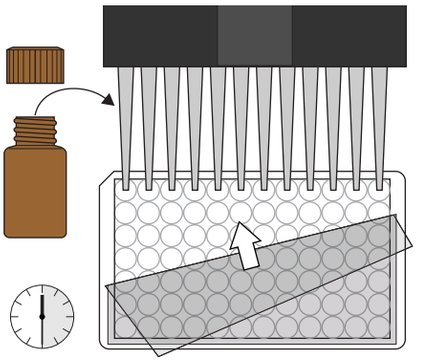
4. Washing of Plates

After incubation, remove the covering and vigorously decant the contents of the wells into a sink. Wash the strips four 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 inverted plate dry on a stack of paper towels.



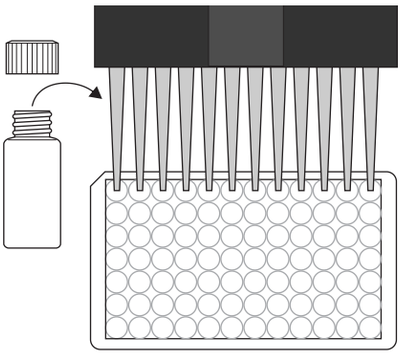
5. Addition of Substrate/Color Solution

Add 150 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 circular motion on the benchtop. Be careful not to spill the contents. Incubate the strips for 30 minutes at room temperature.



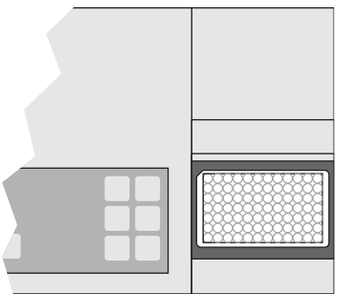
6. Addition of Stopping Solution

Add 100 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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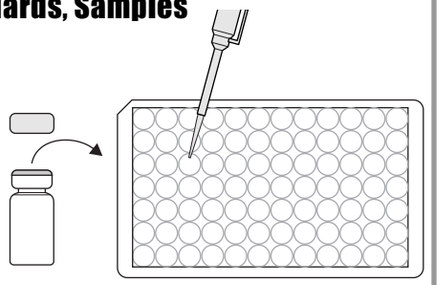
BMAA Plate, Concise ELISA Procedure

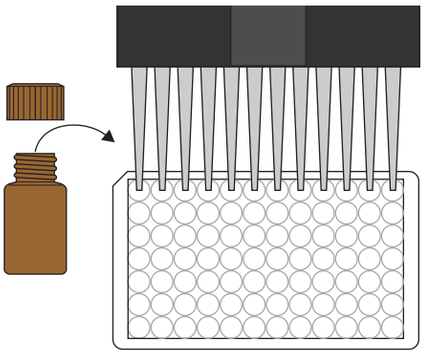
1. Addition of Standards, Samples

Add 100 uL of standard solutions or samples.



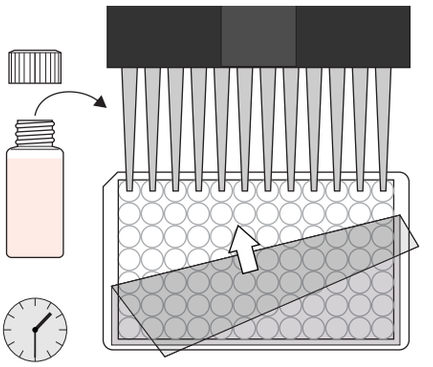
2. Addition of Enyzme Conjugate

Add 50 uL of enzyme conjugate.



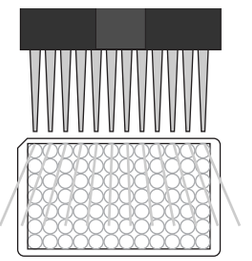
3. Addition of Antibody Solution

Add 50 uL of antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 90 minutes at room temperature.

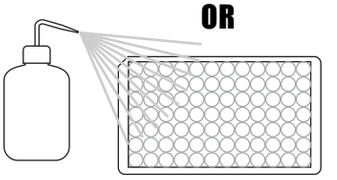


4. Washing of Plates

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

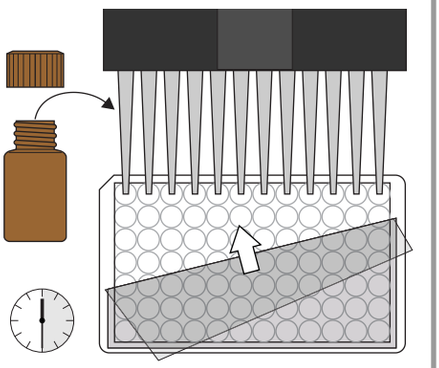


OR



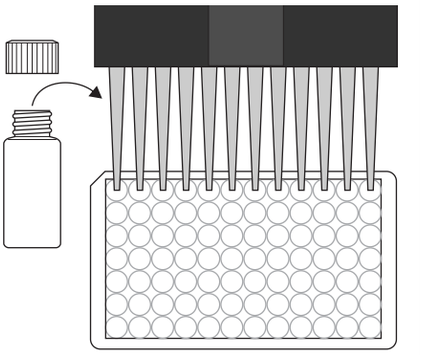
5. Addition of Substrate/Color Solution

Add 150 uL of substrate/color solution. Incubate 30 minutes at room temperature away from direct sunlight.



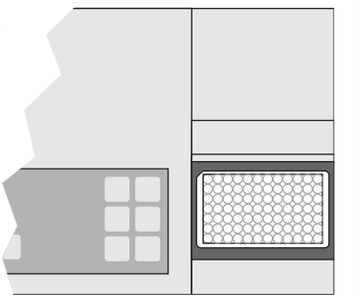
6. Addition of Stopping Solution

Add 100 uL of stop solution.



7. Measurement of Color

Measure absorbance at 450 nm. Calculate results.



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Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: BMAA ELISA Plate Kit
Product Code: 520040
1.2 Identified Use: Determination of BMAA 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.