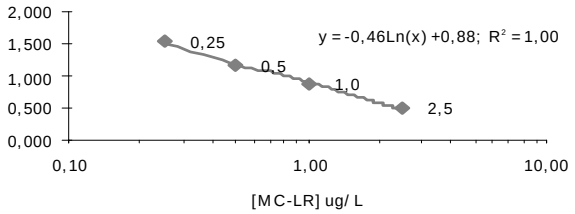
E. Calculations and Graphic Representation of Results

****Note:** A worksheet to calculate the results is available from Abraxis free of charge. Please, contact us for further information.

1 Obtain a standard curve by plotting standards absorbance at 405 nm in the *y axis* and concentration of Microcystin-LR in a logarithmic *x axis*. Draw a standard curve. An example of standard curve is shown below:



2 The concentration of microcystins in the sample is calculated by interpolating the calibration curve or using the following equation:

$$y = a\,Lnx + b \qquad x = EXP(y\text{-}b/a)$$

Where "*x*" value is concentration of microcystin-LR equivalents in the sample and the "*y*" the absorbance at 405 nm.

F. REFERENCES

1. An, J., and W.W. Carmichael. 1994. Use of a colorimetric protein phosphatase assay and enzyme linked immunoassay for the study of microcystins and nodularins. Toxicon. 1994 Dec;32(12):1495-507.
2. McElhiney, J., and Lawton, L.A. Detection of the cyanobacterial hepatotoxins microcystins. Toxicol Appl Pharmacol. 2005 Mar 15;203(3):219-30.
3. WHO (1998) Guidelines for Drinking-Water Quality. Second ed. Addendum to Vol. 1. World Health Organization, Geneva.
4. Bouaicha N, Maatouk I, Vincent G, Levi Y. A colorimetric and fluorometric microplate assay for the detection of microcystin-LR in drinking water without preconcentration. Food Chem Toxicol. 2002 Nov;40(11):1677-83.

For ordering or technical assistance contact:

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ABRAXIS

Microcystins/Nodularins PP2A, Microtiter Plate

Test for the Detection of Microcystins and Nodularins In Water

Product No. 520032

1. General Description

Microcystins/Nodularins PP2A Kit is an enzymatic test for the detection of microcystins and nodularins in water. A simple and rapid method that allows to quantify whether the toxin concentration is over the maximum allowed levels (1 μg/L, OMS 1998).

2. Safety Instructions

The standard solutions in this test kit contain small amounts of Microcystins in solution. Avoid contact of standard and stopping solutions with skin and mucous membranes. If these reagents come in contact with the skin, wash with water. Recommended: Polypropylene material should be avoided throughout sample collection, conservation and treatment, since loss of toxins has been shown to occur.

3. Storage and Stability

The Microcystins/Nodularins PP2A Kit should to be stored in the refrigerator (4–8°C) prior to use and protected from light. 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.

4. Test Principle

Microcystins/Nodularins PP2A Kit is based on the phosphatase activity inhibition by microcystins. Under normal conditions the phophatase is able to hydrolyse a specific substrate that can be detected at 405 nm. Samples containing microcystins will inhibit the enzyme activity proportionally to the amount of toxin contained in the sample. The concentration of the toxin in the sample can be calculated using a standard curve.

5. Limitations of the Microcystins ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water 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 water samples, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C). The assay procedure should be performed away from direct sun light.

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

Working Instuctions

A. Materials Provided

1. Microtiter plate
2. 4 vials of Phosphatase
3. Standards Microcystins (4): 0.25, 0.50, 1.00, 2.50 ppb
4. 1 vial Chromogenic Substrate
5. 1 vial Phosphatase Dilution Buffer
6. 1 vial Stop Soultion

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the assay buffer, 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.

SOLUTIONS

All reagents must be allowed to reach room temperatura (23 ± 3ºC) before starting the assay.

1. Phophatase Solution: Add 3 mL of Phosphatase Dilution Buffer to one of the Phosphatase vials and mix carefully by inversion. Gently shake the solution at room temperature (23 ± 3 ºC) for 60 minutes (or manually several times during that period) to ensure that the enzyme is fully hydrated. This solution must be stored under refrigeration if not used immediately after dissolution. Do not use the Phosphatase Solution for following days.

Each enzyme vial contains enough volume of phosphatase for 24 wells. If more than one vial is needed, dissolve each vial as described above, make a pool with the content of those vials and mix gently by inversion before use.

Attention: this reagent is blue and becomes brownish when dissolved. If the phosphatase turns to brownish colour before the hydratation, please discard as this reagent could be damaged.

2. Standards: the standards are ready to use. They are provided in vials containing a total volume of 1.2 mL.

C. Assay Procedure

1. Add 50 µL of each Microcystin-LR standard in duplicate (i. e.: wells A1 and A2, 0.25 µg/L; wells B1 and B2, 0.50 µg/L; wells C1 and C2, 1.00 µg/L; wells D1 and D2, 2.50 µg/L). We recommend using duplicates or triplicates.

2. Add 50 µL of each sample in duplicate into the remaining wells of the microtiter plate.

3. Add 70 µL of the Phosphatase Solution to each well.

4. Add 90 µL of Chromogenic Substrate to each well and mix gently. The substrate contains solid in suspension. Do not mix the reagent prior to use and avoid taking any solid.

5. Put the adhesive film on wells and Incubate the plate for 30 minutes at 37 ºC.

6. Add 70 µL of Stop Solution to each well. Mix gently.

7. Read the absorbance of samples and standards at 405 nm. Use an empty well as blank, if necessary.

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

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wave length 405 nm)
4. Timer
5. Tape or Parafilm
6. Glass vials with Teflon-lined caps
7. Distilled or deionized water
8. Vortex mixer
9. Heater at 37 +/- 2 °C

E. Sample Preparation

1. Drinking water (water treated in drinking water stations):

Sample preparation is not required. Following the procedure described in section C (Assay Procedure) the content of dissolved microcystins will be determined. Please, pay attention to the note mentioned below.

2. Water from reservoirs, rivers, etc

The content of dissolved microcystins, intracellular microcystins and total microcystins can be determined (see Scheme on next page). Please, pay attention to the note mentioned below.

2.1 Dissolved microcystins: Sample preparation is not required. Following the procedure described in section C, dissolved microcystins content will be determined.

If any interferences are suspected (i.e. high concentration of heavy metals, turbidity), please contact Abraxis for technical assistance.

2.2 Intracellular microcystins:

a) Take 200 mL of sample and filer in vacuum through a 0.8 µm nylon membrane (i.e. Whatman Nylon Membrane Filters, ref.: 7408-004). Reserve the filtrate for further determination of total microcystins, as it is explained in paragraph 2.3.

b) Take the membrane with the residue and place in a glass flask. The membrane can be cut into pieces to improve the extraction step.

c) Add 10 mL of 80% MeOH in water with 0.1% TFA and 0.1% Tween 20. Incubate at room temperature for 30 minutes with gentle stirring and in absence of light.

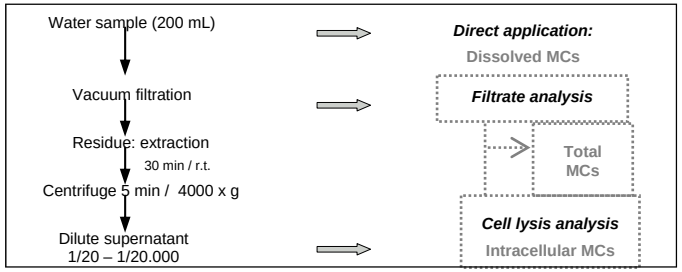
d) Centrifuge at 4000 g for 5 min.

e) Take the supernatant and dilute it 20 times (dilution 1/20) with distilled water. At this point, the sample is ready to continue the assay as is shown in Section C. This way, the content of intracellular microcystins is determined. If the concentration of MC-LR equivalents exceeds 2.5 µg/L, we advice to perform the assay on a range of supernatant dilutions of 1/20, 1/200, 1/2000,…

2.3 Total microcystins:

Use the filtrate obtained in 2.2.a. and perform the assay described in section C. Total microcystins contained in the sample are calculated by adding the concentration of microcystins found in the filtrate plus the intracellular microcystins (2.2.e).

NOTE: Presence of thiosulphate (or strong oxidizing reagents) may interfere in the assay, therefore collecting samples in bottles with this chemical or adding it to the simple prior to testing should be avoided.



Scheme procedure for water samples from reservoirs, rivers, etc.

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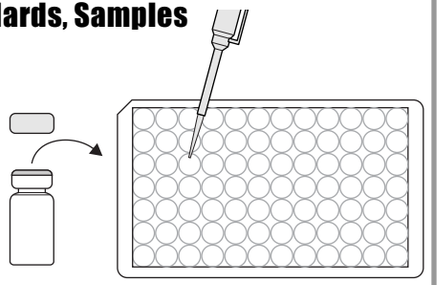
Microcystin PP2A Plate Kit, Detailed Procedure

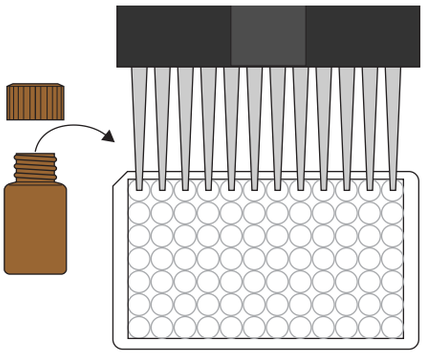
1. Addition of Standards, Samples

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



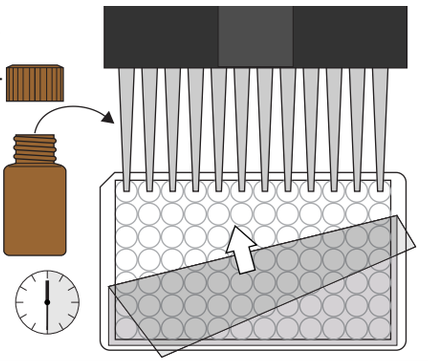
2. Addition of Phosphatase Solution

Add 70 uL of the Phosphatase solution to the individual wells successively using a multi- channel pipette or a stepping pipette.



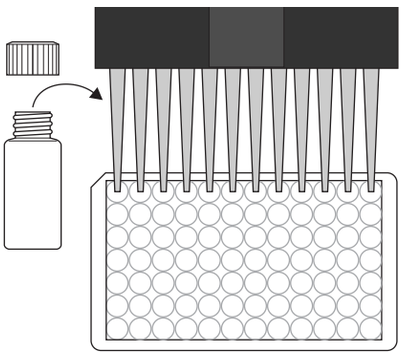
3. Addition of Chromogenic Substrate

Add 90 uL of the Chromogenic Substrate 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 37ºC.



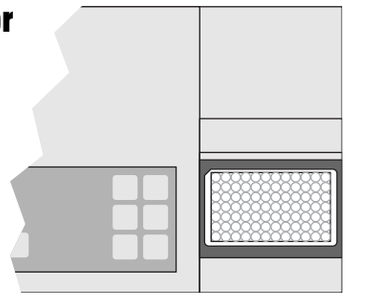
4. Addition of Stopping Solution

Add 70 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.



5. Measurement of Color

Read the absorbance at 405 nm using a microplate ELISA reader. Calculate results.



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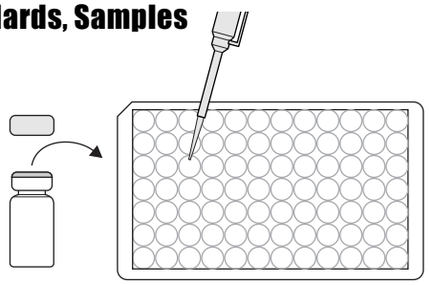
Microcystin PP2A Plate Kit, Concise Procedure

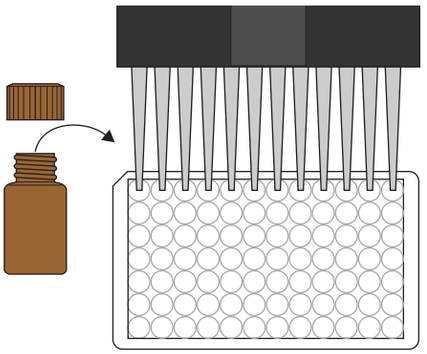
1. Addition of Standards, Samples

Add 50 uL of standard solutions, and samples.



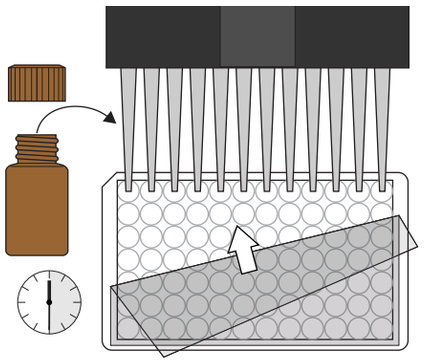
2. Addition of Phosphatase Solution

Add 70 uL of the Phosphatase solution.



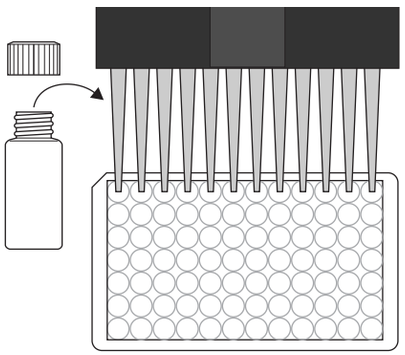
3. Addition of Chromogenic Substrate

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



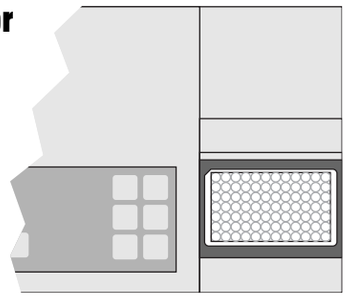
4. Addition of Stopping Solution

Add 70 uL of Stopping Solution.



5. Measurement of Color

Read the absorbance at 405 nm using a microplate ELISA reader. Calculate results.



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Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: Microcystins/Nodularins PP2A Plate Kit, Microcystins/Nodularins PP2A Tube Kit
Product Code: 520032, 520033
1.2 Identified Use: Determination of Microcystins/Nodularins 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 of the mixture
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: No data available
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: No data available

Eye contact: No data available
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

SARA Title III, Section 302 Components: No chemicals in this material are subject to the reporting requirements
SARA Title III, Section 313 Components: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.
SARA 311/312 Hazards: No SARA hazards
State Right-to-Know
Massachusetts: No components are subject to the Massachusetts Right to Know Act.
Pennsylvania: Disodium 4-nitrophenyl phosphate, CAS No. 4262-83-9
New Jersey: Disodium 4-nitrophenyl phosphate, CAS No. 4262-83-9
California Prop. 65 Components: This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

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.