

## E. Calculations of Results

Use the following table to determine the level of Microcystins in sample:

Sample Absorbance	MC-LR equivalents
$A_s > A_{0.5}$	< 0.5 µg/L
$A_s > A_{1.0}$	< 1.0 µg/L
$A_s > A_{2.5}$	< 2.5 µg/L
$A_s < A_{2.5}$	> 2.5 µg/L

$A_s$ : Sample Absorbance.  
 $A_{0.5}$ : 0.5 µg/L Standard Absorbance.  
 $A_{1.0}$ : 1.0 µg/L Standard Absorbance.  
 $A_{2.5}$ : 2.5 µg/L Standard Absorbance.

Example of results interpretation:

Standard/Sample	Absorbance 405 nm	MC-LR equivalents
0.5 ng/mL	1.62	N/A
1.0 ng/mL	1.23	N/A
2.5 ng/mL	0.68	N/A
Sample 1	1.83	sample < 0.5 µg/L
Sample 2	0.75	1.0 µg/mL < sample < 2.5 µg/L

N/A: No Aplicable

## F. REFERENCES

- 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. *Toxicol.* 1994 Dec;32(12):1495-507.
- McElhiney, J., and Lawton, L.A. Detection of the cyanobacterial hepatotoxins microcystins. *Toxicol Appl Pharmacol.* 2005 Mar 15;203(3):219-30.
- WHO (1998) Guidelines for Drinking-Water Quality. Second ed. Addendum to Vol. 1. World Health Organization, Geneva.
- 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.

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## Microcystins/Nodularins PP2A, Tube Kit

Test for the Detection of Microcystins and Nodularins In Water

**Product No. 520033**

### 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 phosphatase 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 Instructions

### A. Materials Provided

1. 32 Test Tubes
2. 4 vials of Phosphatase
3. Standards Microcystins (3): 0.5, 1.0, 2.5 ppb
4. 1 vial Chromogenic Substrate
5. 1 vial Phosphatase Dilution Buffer
6. 1 vial Stop Solution

### 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 incubation 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 temperature ( $23 \pm 3^\circ\text{C}$ ) before starting the assay.

1. Phosphatase Solution: Add 1 mL of Phosphatase Dilution Buffer to one of the Phosphatase vials and mix carefully by inversion. Gently shake the solution at room temperature ( $23 \pm 3^\circ\text{C}$ ) for 30 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 8 tubes. 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 hydration, 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

Following the assay procedure described below, semiquantitative results will be obtained. If a quantitative analysis is required, the procedure can be easily adapted by testing standards and samples in duplicate. **Please, contact Abraxis and we will provide you with a worksheet to calculate the results (info@abraxiskits.com).**

1. Add 250  $\mu\text{L}$  of Microcystin-LR standards into three tubes (i. e.: tube 1, standard of 0.5  $\mu\text{g/L}$ ; tube 2, standard of 1.0  $\mu\text{g/L}$ ; tube 3, standard of 2.5  $\mu\text{g/L}$ ).
2. Add 250  $\mu\text{L}$  of the samples into the remaining tubes.
3. Add 100  $\mu\text{L}$  of the Phosphatase Solution to each tube.
4. Add 350  $\mu\text{L}$  of Chromogenic Substrate to each tube and mix gently. The substrate contains solid in suspension. Do not mix the reagent prior to use and avoid taking any solid.
5. Incubate for 30 minutes at  $37^\circ\text{C}$  (tubes should be covered with aluminium foil to prevent evaporation).
6. Add 500  $\mu\text{L}$  of Stop Solution to each tube. Mix gently.
7. Read Absorbance at 405 nm. Use an empty tube/cuvette 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  $\mu\text{L}$ )
2. Multi-channel pipette (100-1,000  $\mu\text{L}$ ) or stepper pipette with plastic tips (100-1,000  $\mu\text{L}$ )
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  $37 \pm 2^\circ\text{C}$

### 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 below). 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.\*\*\***

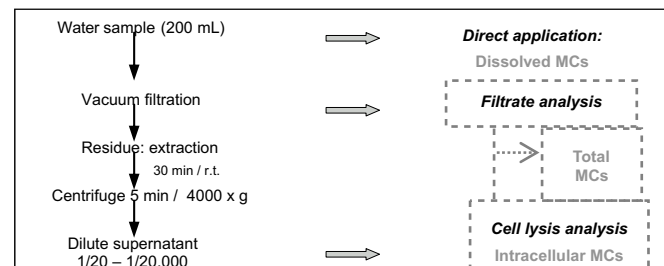
#### 2.2 Intracellular microcystins:

- a) Take 200 mL of sample and filter in vacuum through a 0.8m 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 g/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 sample prior to testing should be avoided.



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



# 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](mailto: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

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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.

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