

## Okadaic Acid (PP2A), Microtiter Plate Test for the Detection of Okadaic Acid-toxins group

Product No. 520025

$$x = \text{EXP} (y - b/a)$$

Where *x* is the OA concentration in the sample (Cs) and *y* the absorbance of the sample.

Note: An Excel worksheet to calculate results is available upon request.

3.- Calculate the diarrhetic shellfish toxins concentration in tissue (Ct) as follows:

$$Ct (\mu\text{g/kg}) = \frac{(Cs \text{ (nM)} \times FD \times MW \text{ (g/mol)}) \times Ve \text{ (L)}}{Mt \text{ (g)}}$$

Ct: toxins concentration in tissue, expressed as equivalents of OA; Cs: toxins concentration in sample; FD: Methanolic extract dilution factor (i.e. 640  $\mu\text{L}/20 \text{ mL} \rightarrow \times 31.25$ ); MW: Okadaic acid molecular weight = 805; Ve: Methanolic extract volume (0.025L); Mt: Tissue weight (5g).

Example: for OA concentration of 1.5 nM:  $1.5 \text{ nM} \times 31.25 \times 805 \text{ g/mol} \times 0.025 \text{ L} / 5 \text{ g} = 189 \mu\text{g OA q/kg}$ .  
For samples with OA concentration falling outside the working range (< 0.5 nM or > 2.8 nM), results will be reported as < 0.5 nM (or < 63  $\mu\text{g/Kg}$ ) or > 2.8 nM (or > 352  $\mu\text{g/kg}$ ), respectively.

### F. Importance of Okadaic Acid Determination

Okadaic Acid is one of the "diarrhetic shellfish poisons" (DSP) produced by the dinoflagellate species *Dinophysis* and *Prorocentrum*. Contamination of shellfish with okadaic acid has been associated with harmful algal blooms throughout the world.

In man, DSP causes dose-dependent symptoms of diarrhea, nausea, and vomiting. The action level established by the FDA is 0.2ppm. The EU has established a level of 160 $\mu\text{g}$  OA eq (OA, DTXs, PTXs)/kg.

The Okadaic Acid Phosphatase assay allows the determination of 40 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 1 hour.

### G. REFERENCES

1. A Fluorescent Microplate Assay for Diarrhetic Shellfish Toxins. Vиейtes M. R. et al. Analytical Biochemistry 248, 258-264 (1997).
2. Protein phosphatase inhibition assay adapted for determination of total DSP in contaminated mussels. Mounfort D. O. et al. Toxicon 39, 383-390 (2001).
3. Inter-laboratory validation of the fluorescent protein phosphatase inhibition assay to determine diarrhetic shellfish toxins: intercomparison with liquid chromatography and mouse bioassay. Gonzalez J. C., et al. Analytica Chimica Acta 466, 233-246 (2002).

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For ordering or technical assistance contact:

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### 1. General Description

This protocol specifies a method for the quantitative determination of Okadaic Acid (OA) and other carboxylic toxins of the OA group including DTX1, DTX2 and DTX3 by a colorimetric phosphatase inhibition assay. This method is applicable to shellfish species such as mussels, clams, oysters and scallops.

### 2. Safety Instructions

The standard solutions in this test kit contain small amounts of Okadaic Acid 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 Okadaic Acid-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

Test based on the phosphatase activity inhibition by OA-toxins group, responsible for diarrhetic shellfish poisoning (DSP). Phosphatase enzyme PP2A is able to hydrolyse a specific substrate, yielding a product that can be detected at 405 nm. Samples containing toxins from the okadaic acid group will inhibit the enzyme activity proportionally to the amount of toxin contained in the sample. The concentration of toxin in the sample can be calculated using a standard curve.

### 5. Limitations of the Okadaic Phosphatase Assay, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in 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 assay and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 40°C). The assay procedure should be performed away from direct sun light.

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

## Working Instructions

### A. Materials Provided

1. Microtiter plate
2. Phosphatase, 4 vials
3. Standards Okadaic Acid (5): 0.5, 0.8, 1.2, 1.8, and 2.8 nM
4. Chromogenic Substrate, 1 vial
5. Phosphatase Dilution Buffer, 1 vial
6. Stock Buffer Solution, 1 vial
7. Stop Solution, 1 vial
8. Adhesive Film

### B. 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 (50-250  $\mu\text{L}$ ) or stepper pipette with plastic tips (10-250  $\mu\text{L}$ )
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  $30 \pm 2$  °C
10. Water bath at  $76 \pm 2$  °C
11. Methanol (analytical grade)
12. NaOH, 2.5 N (analytical grade)
13. HCl, 2.5N (analytical grade)
14. Deionized water (grade 2, ISO3696)
15. Graded 50 mL centrifuge tubes with screw caps
16. Tube shaker

### C. 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

- 1.- **Okadaic Acid Standards:** to make sure these solutions are homogeneous, it is very important to mix well using a vortex, before applying to the plate.
- 2.- **Chromogenic Substrate solution:** The solution contains stabilization resin. Make sure this resin is not added to the microwells. To assure that, it is recommended to transfer the volume needed into a transparent labware (i.e.: test tube or eppendorf) and take the solution from that container to add into the wells. *Note:* Do not use this solution if the absorbance of 90  $\mu\text{L}$  of this solution at 405 nm is over 0.6.
- 3.- **Phosphatase solution:** Add 2.0 mL of phosphatase dilution buffer (*Phosphatase Dilution Buffer*) to one of the phosphatase vials (*Phosphatase*) and dissolve by mixing gently for 1 hour  $\pm$  5 minutes at room temperature ( $22 \pm 2$  °C) to ensure that the enzyme is fully hydrated. Do not use the tube shaker at any moment. This solution must be stored under refrigeration if not in use immediately after preparation. Do not use the phosphatase solution for following days. Each enzyme vial contains enough volume for 24 wells. If more than one vial is used in the assay, dissolve each vial as described above, make a pool with the content of the vials and mix gently, by inversion, before use.  
**\*Attention:** this reagent is blue and becomes brownish when dissolved. If brownish colour is noticed before hydration, discard this reagent as it could be damaged.
- 4.- **Buffer solution x1:** dilute the *Stock Buffer Solution* included in the kit by mixing 1 volume with 9 volumes of deionized water. Use buffer solution x1 only freshly made, and store under refrigeration if not in use immediately after preparation.
- 5.- **2.5 N NaOH:** weigh 100 g of NaOH and add 500 mL of water and dissolve. Transfer to a volume-tric flask and add deionized water up to a final volume of 1000 mL.
- 6.- **2.5 N HCl:** add 205 mL of HCl (37 %) to 400 mL of deionised water already contained in a volumetric flask. Make the volume up to 1000 mL with deionized water.

## D. Assay Procedure

### Warning:

The volume of some reagents used in this assay is small and special attention must be paid when added to the wells:

- Make sure the pipettes are calibrated before running the assay.
- Use pipettes according to the volumes to be dispensed. Use pipettes with a maximum pipette volume of 100 or 200  $\mu\text{L}$ .
- Be sure that the incubator's temperature is stabilized before use.

It is recommended to run samples and standards in duplicate.

- 1.- Add 50  $\mu\text{L}$  of samples or standards.
- 2.- Add 70  $\mu\text{L}$  of the Phosphatase Solution to each well. Mix well by gentle tapping on the side of the plate.
- 3.- Cover the plate with the adhesive film provided and incubate for  $20 \pm 0.5$  minutes at  $30 \pm 2$  °C.
- 4.- Remove the adhesive film and add 90  $\mu\text{L}$  of Chromogenic Substrate to each well. Mix well by gently tapping on the side of the plate.
- 5.- Cover the plate with the adhesive film and incubate  $30 \pm 0.5$  minutes at  $30 \pm 2$  °C.
- 6.- Remove the adhesive film and add 70  $\mu\text{L}$  of Stop Solution to each well.
- 7.- Read absorbance of samples and standards at 405 nm.

### E. Sample Preparation

The method described below includes a hydrolysis step to detect all toxins forms of okadaic acid (okadaic acid and dinophisistoxins).

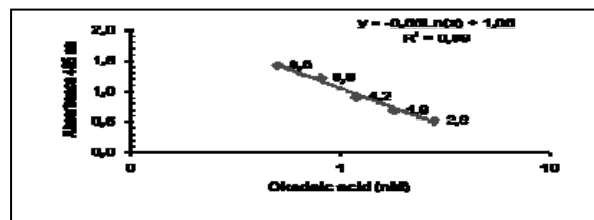
- 1.- Clean the shell thoroughly using water
- 2.- Open the shellfish by cutting the adductor muscles.
- 3.- Wash inside the shell thoroughly to get rid of any dirt.
- 4.- Remove the tissue inside the shell by cutting all the muscles attached to the shell.
- 5.- Place the shellfish tissue in a filter paper for few minutes to remove water in excess.

It is recommended to use graded 50 mL centrifuge tubes with screw caps during the following steps of hydrolysis in order to prevent losses due to labware changes.

- 6.- Mash the shellfish tissue to obtain a representative sample and weigh 5 g. Add 25 mL of Methanol and homogenise the mixture for 2 minutes using a tube shaker.
- 7.- Centrifuge at 2000 g for 10 min at 4 °C. The supernatant (*methanolic extract*) is poured into a centrifuge tube.
- 8.- Take 640  $\mu\text{L}$  of *methanolic extract* and pour into another centrifuge tube.
- 9.- Add 100  $\mu\text{L}$  of 2.5 N NaOH.
- 10.- Seal and heat at  $76 \pm 2$  °C for 40 minutes.
- 11.- Add 80  $\mu\text{L}$  of 2.5 N HCl (the sample does not need to be cooled down previously).
- 12.- Add up to 20 mL of Buffer solution x1.

### E. Calculations and Graphic Representation of Results

- 1.- Obtain a standard curve by plotting the absorbance values in a linear *y axis* and the concentration of okadaic acid in a logarithmic *x axis* and use a logarithmic fitting as shown in the graphic next page.  $R^2$  has to be greater than or equal to 0.96.

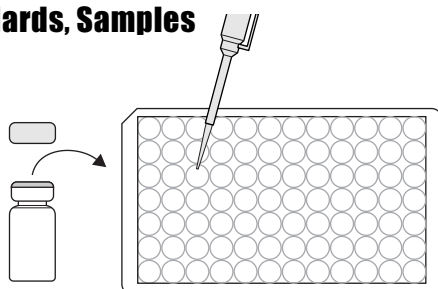


- 2.- The OA concentration contained in the sample ( $C_s$ ) is calculated by interpolation into the calibration curve or using the following equation:

# Okadaic Acid (DSP) PP2A Plate Kit, Detailed Procedure

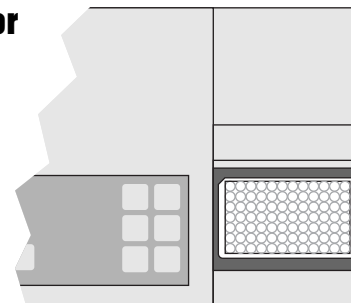
## 1. Addition of Standards, Samples

Add 50  $\mu$ L 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.



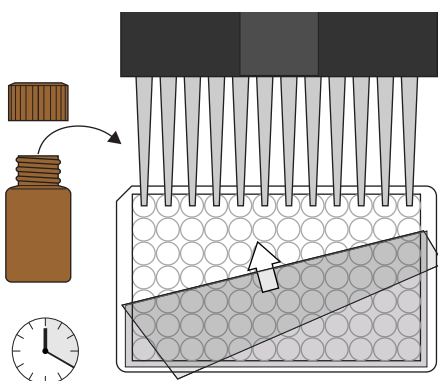
## 5. Measurement of Color

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



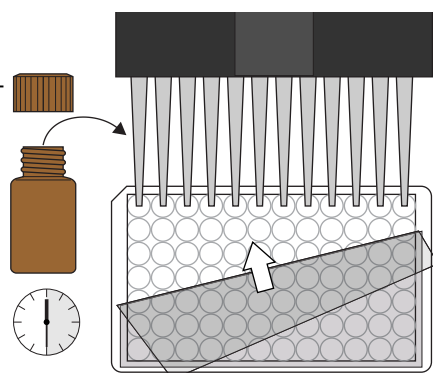
## 2. Addition of Phosphatase Solution

Add 70  $\mu$ L of the Phosphatase solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Incubate the strips for 20 min at 30°C.



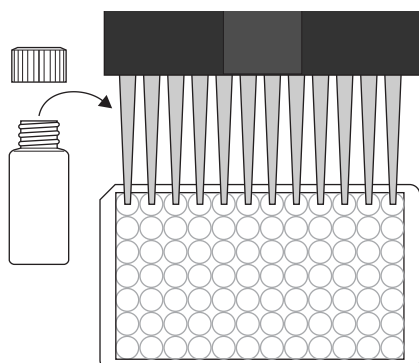
## 3. Addition of Chromogenic Substrate

Add 90  $\mu$ L 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 30°C.



## 4. Addition of Stopping Solution

Add 70  $\mu$ L of stop solution to the wells in the same sequence as for the substrate solution using a multi-channel pipette or a stepping pipette.



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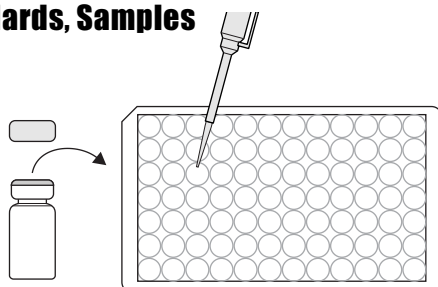
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# Okadaic Acid (DSP) PP2A Plate Kit, Concise Procedure

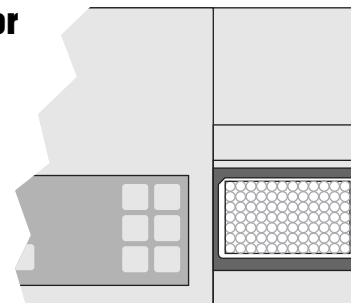
## 1. Addition of Standards, Samples

Add 50  $\mu$ L of standard solutions, and samples.



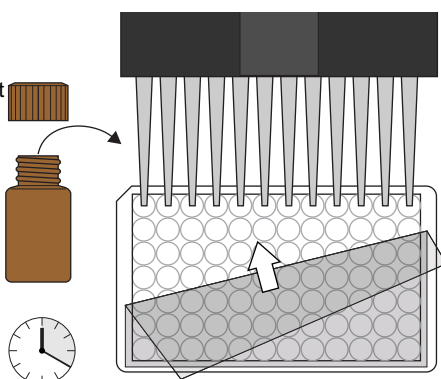
## 5. Measurement of Color

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



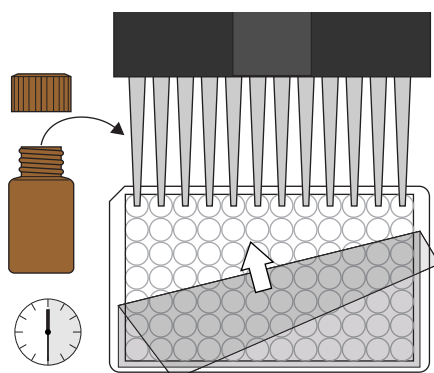
## 2. Addition of Phosphatase Solution

Add 70  $\mu$ L of the Phosphatase solution. Incubate for 20 minutes at 30°C.



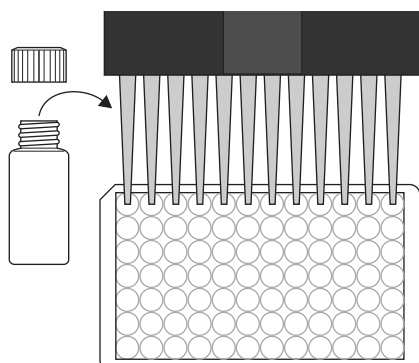
## 3. Addition of Chromogenic Substrate

Add 90  $\mu$ L of Chromogenic Substrate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at 30°C.



## 4. Addition of Stopping Solution

Add 70  $\mu$ L of Stopping Solution.



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# Safety Data Sheet

## Section 1: Product and Company Identification

### 1.1 Product Identifiers:

**Product Name:** Okadaic Acid (DSP) PP2A and Okadaic Acid (DSP) (EC 2002/225 Compliant) ELISA Plate Kits

**Product Code:** 520021, 520025

**1.2 Identified Use:** Determination of Okadaic Acid (DSP) 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

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:** 3

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