

Sulfamethazine

• Intended Use

For the detection and quantitation of sulfamethazine in water (groundwater, surface water, well water). For other use contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Sulfamethazine Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of sulfamethazine. In the assay system, standards or samples to be tested are added, along with an enzyme conjugate, and an antibody specific for sulfamethazine, to microtiter wells coated with Goat anti-rabbit antibody. At this point a competitive reaction occurs between the sulfamethazine which may be in the sample and the enzyme labeled sulfamethazine for the antibody binding sites. The reaction is allowed to incubate for sixty (60) minutes. At the end of the incubation period, the wells are washed with Washing Buffer.

The presence of sulfamethazine is detected by adding the "Color Solution", which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled sulfamethazine bound to the sulfamethazine antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After a thirty (30) minute incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled sulfamethazine (conjugate) was in competition with the unlabeled sulfamethazine (sample) for the antibody sites, the **color developed is inversely proportional to the concentration of sulfamethazine in the sample.**

• Reagents

The Abraxis Sulfamethazine Plate Kit contains the following items:

- 1. Microtiter Plate coated with Goat-Anti Rabbit Antibody**
96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).
- 2. Sulfamethazine Antibody Solution**
Sulfamethazine antibody (rabbit anti-Sulfamethazine) solution in a buffered saline solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL
- 3. Sulfamethazine Enzyme Conjugate**
Horseradish peroxidase (HRP) labeled Sulfamethazine analog in a buffered solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL
- 4. Sulfamethazine Standards**
Four concentrations (0, 0.05, 0.15, 0.25, 0.50, 1.5, 5.0 ppb) of Sulfamethazine standards in distilled water with preservative and stabilizers.
96 test kit: Each vial contains 1.0 mL
- 5. Diluent/Zero Standard (Sample Diluent)**
Distilled water with preservative and stabilizers without any detectable Sulfamethazine.
96 test kit: One bottle containing 30 mL
- 6. Color Solution**
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.
96 test kit: One bottle containing 12 mL
- 7. Stopping Solution**
A solution of diluted acid.
96 test kit: Two bottles containing 6 mL each
- 8. Washing Buffer (5x) Concentrate**
Buffered salts with detergent and preservatives.
96 test kit: One bottle containing 100 mL

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Precision pipets capable of delivering 50, 75, 100, 150, and 250 μ L, and tips*

Tape or Parafilm®*

Timer*

Distilled or deionized water for diluting Wash Buffer

Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*

Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered (e.g. 0.2 μ m Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the Sulfamethazine concentration of a sample exceeds 5.0 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 μ L of the sample to 900 μ L of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor, e.g. 10.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of H₂O). This solution is used to wash the antibody coated wells.

• Procedural Notes and Precautions

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

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

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

The microtiter plate consists of 12 strips of 8 wells. If fewer than twelve strips are used, remove the unneeded strips and store refrigerated in the resealable foil bag (with desiccant) provided.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis Sulfamethazine Assay will detect sulfamethazine and other sulfonamides to different degrees. Refer to specificity table for data on several of the sulfonamides. The Abraxis Sulfamethazine Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St6: Standards
S1-Sx: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S4	S2									
B	S1	S4	S2									
C	S1	S5	S2									
D	S1	S5	S2									
E	S1	S4										
F	S1	S4										
G	S1	S1										
H	S1	S1										

1. Add 50 μ L of the appropriate standard or sample. Analysis in duplicates or triplicates is recommended.
2. Add 50 μ L of Sulfamethazine Enzyme Conjugate.
3. Add 50 μ L of Sulfamethazine antibody solution successively to each well. Cover wells with parafilm or tape to prevent contamination and evaporation. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents. Incubate at ambient temperature for 60 minutes.
4. After the incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips with the diluted Wash Buffer (see Reagent Preparation) by adding a volume of at least 250 μ L of Wash Buffer to each well. Vigorously shake the contents of the wells into a waste container. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels. Repeat this wash step two times, for a total of 3 rinses.

- Add 100 µL of Color Solution successively to each well. Cover wells with parafilm or tape. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Incubate at ambient temperature for 30 minutes.
- Add 50 µL of Stopping Solution successively to each well.
- Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

B/Bo Compound	50% (ppb)	Cross-Reactivity (%)
Sulfamethazine	0.6	100
Sulfacetamide	0.6	100
Sulfamerazine	4.0	15
Sulfathiazole	4.1	15

The following sulfa drugs gave cross-reactivity of < 1%: sulfasalazine, sulfasomidin, sulfisoxazole, sulfiphenazole, sulfaguandine, sulfachloropyridazine, sulfaquinoxaline, sulfadiazine, sulfamethoxyypyridazine, sulfameter, sulfadimethoxine, sulfamilamide, sulfapyridine, sulfabenzamide, sulfamethoxazole.

The following compounds demonstrated no reactivity in the Abraxis Sulfamethazine Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

• Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameter or alternatively point to point). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Diluent/Zero Standard (Standard 0). Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (Y) axis versus the corresponding Sulfamethazine concentration on the horizontal log (X) axis on the graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of Sulfamethazine (in ppb) by interpolation using the constructed standard curve.

Samples exhibiting a concentration lower than 0.05 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 5.0 ppb must be diluted to obtain accurate results.

• Expected Results

In a study with water samples from locations across the U.S., the Abraxis Sulfamethazine Assay was shown to correlate well with another commercial Sulfamethazine immunoassay.

• Performance Data

Precision

The following results were obtained:

Control	0.15	0.50	1.5
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.156	0.52	1.37
% CV (within assay)	10.8	10.4	8.7
% CV (between assay)	15.6	11.8	10.9

Sensitivity

The Abraxis Sulfamethazine Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.04 ppb.

Recovery

Five (5) groundwater samples, were spiked with various levels of sulfamethazine and then assayed using the Abraxis Sulfamethazine Assay. The following results were obtained:

Amount of Sulfamethazine Added (ppb)	Recovery		
	Mean (ppb)	S.D. (ppb)	%
0.25	0.29	0.05	117
1.0	0.94	0.05	94
2.5	1.99	0.08	80
Average			97

Specificity

The cross-reactivity of the Abraxis Sulfamethazine Assay for various sulfonamides can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required for 50% absorbance inhibition (50% B/Bo).

• Ordering information

Abraxis Sulfamethazine Assay Kit 96T PN 515006

• Assistance

For ordering or technical assistance contact:

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

Email: customerservice@lifetechindia.com, www.atzlabs.com; www.lifetechindia.com

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

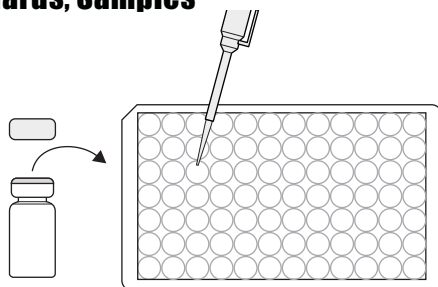
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SULFAMETHAZINE Plate, Detailed ELISA Procedure

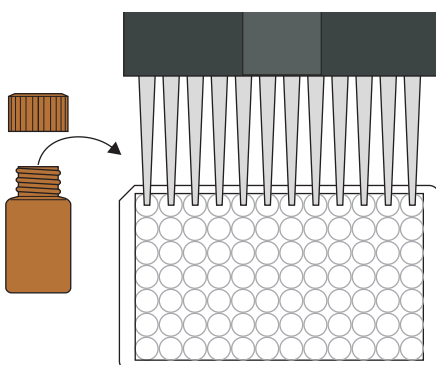
1. Addition of Standards, Samples

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



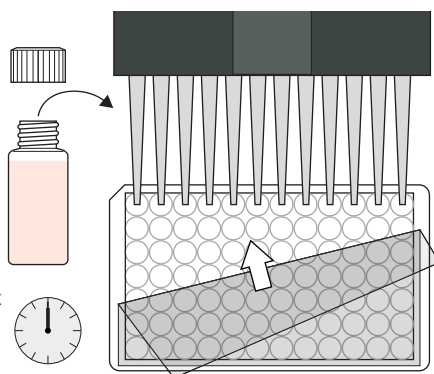
2. Addition of Enzyme Conjugate

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



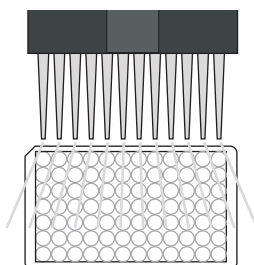
3. Addition of Antibody Solution

Add 50 μ L of the Sulfamethazine 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 rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 60 minutes at room temperature.



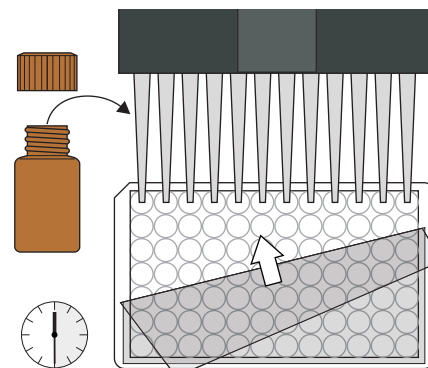
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least 250 μ L of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



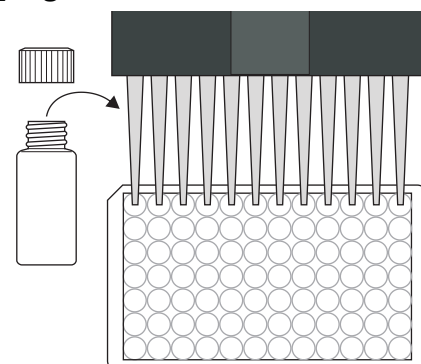
5. Addition of Substrate/Color Solution

Add 100 μ L 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 rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 minutes at room temperature away from direct sunlight.



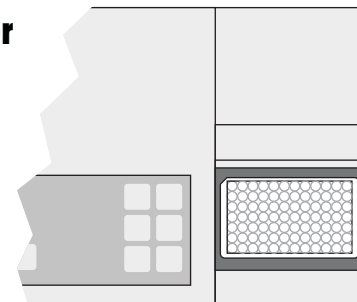
6. Addition of Stopping Solution

Add 50 μ 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.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader within 15 minutes. Calculate results.



India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

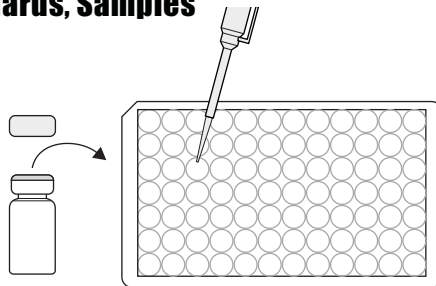
Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

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SULFAMETHAZINE Plate, Concise ELISA Procedure

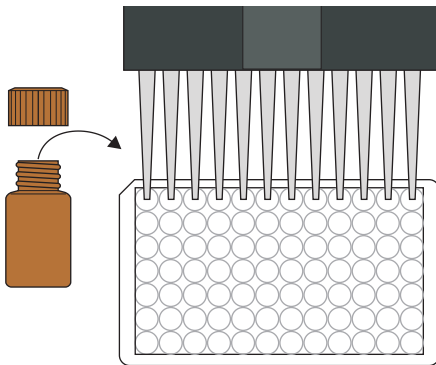
1. Addition of Standards, Samples

Add 50 μ L of standard solutions, samples or sample extracts.



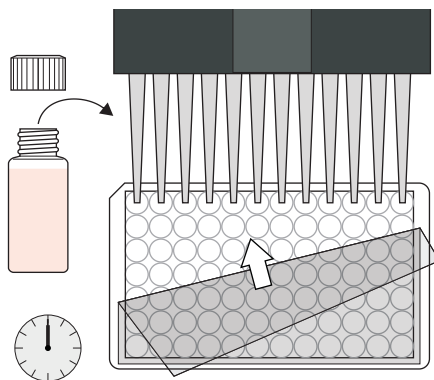
2. Addition of Enzyme Conjugate

Add 50 μ L of enzyme conjugate.



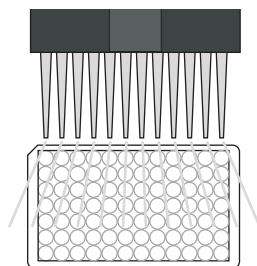
3. Addition of Antibody Solution

Add 50 μ L of the antibody solution. Cover and mix for 30 seconds by moving strip holder in a circular motion on benchtop. Incubate for 60 minutes at room temperature.



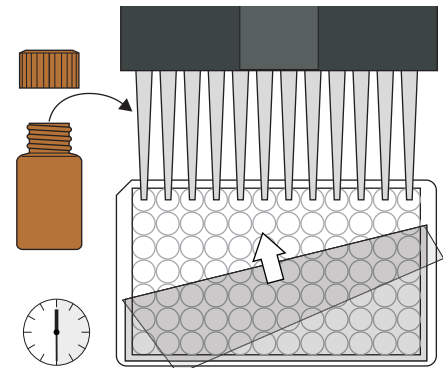
4. Washing of Plates

Wash the wells three times with 250 μ L of diluted 1X washing buffer.



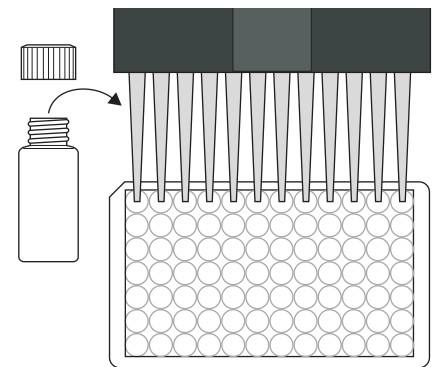
5. Addition of Substrate/Color Solution

Add 100 μ L of substrate/color solution. Cover and mix for 30 seconds by moving strip holder in a circular motion on benchtop. Incubate 30 minutes at room temperature away from direct sunlight.



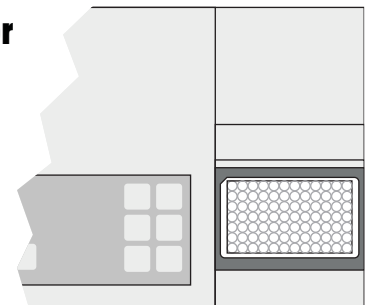
6. Addition of Stopping Solution

Add 50 μ L of stop solution.



7. Measurement of Color

Measure color at 450 nm within 15 minutes. Calculate results.



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Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:

Product Name: Sulfamethazine (SMZ) Magnetic Particle Kit, Sulfamethazine (SMZ) Plate Kit, and Sulfamethoxazole (SMX) Plate Kit

Product Code: 515001, 515006, 522003

1.2 Identified Use: Determination of Sulfamethazine or Sulfamethoxazole 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: Not applicable

Evaporation rate: No data available

Flammability (solid, gas): No data available

Upper/lower flammability or explosive limits: No data available

Vapor pressure: No data available

Vapor density: No data available

Relative density: No data available

Water solubility: Various

Partition coefficient: n-octanol/water: No data available

Auto-ignition temperature: Not applicable

Decomposition temperature: No data available

Viscosity: No data available

Explosive properties: No data available

Oxidizing properties: No data available

9.2 Other information: No data available

Section 10: Stability and Reactivity

10.1 Reactivity: No data available

10.2 Chemical stability: Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions: No data available

10.4 Conditions to avoid: No data available

10.5 Incompatible materials: No data available

10.6 Hazardous decomposition products: No data available. In the event of fire: see section 5.

Section 11: Toxicological Information

11.1 Information on toxicological effects

Acute toxicity: Not available. To the best of our knowledge, the chemical, physical, and toxicological properties of this product have not been thoroughly investigated.

Inhalation: No data available **Ingestion:** No data available

Skin contact: Irritant to skin and mucous membranes.

Eye contact: May cause eye irritation in susceptible persons.

Respiratory or skin sensitization: No data available

Aspiration hazard: No data available

Mutagenicity: No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Teratogenicity: No data available

Reproductive/fertility toxicity: No data available

Specific target organ toxicity, single exposure: No data available

Specific target organ toxicity, repeated exposure: No data available

Section 12: Ecological Information

12.1 Toxicity: No data available

12.2 Persistence and degradability: No data available

12.3 Bioaccumulative potential: No data available

12.4 Mobility in soil: No data available

12.5 Results of PBT and vPvB assessment: No data available

12.6 Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13: Disposal Considerations

13.1 Waste treatment methods

Product: All waste must be handled and disposed according to local, state, and federal regulations. Avoid disposing large volumes in sewer.

Contaminated packaging: All waste must be handled and disposed according to local, state, and federal regulations.

Refer to sections 7 and 8 for safe handling guidance.

Section 14: Transport Information

UN Number: Not regulated

UN Proper shipping name: Not classified as dangerous in the meaning of transport regulations.

Transport hazard class(es): No data available

Packing group: No data available

Environmental hazard: No data available

Bulk transport: No data available

Special considerations: No data available

Section 15: Regulatory Information

To the best of our knowledge, this product contains no substances which, at their given concentrations, are considered hazardous by other regulatory agencies. Refer to section 3.

Section 16: Other information

This information is based on our present knowledge. While Abraxis, Inc. believes that the data contained herein are factual and the opinions expressed represent a best effort to present accurate information, the data are not to be taken as a warranty or representation for which Abraxis, Inc. assumes legal responsibility. The information shall not be taken as being all-inclusive and is to be used only as a guide. The data are offered solely for the user's consideration, investigation, and verification. These suggestions should not be confused with either state, municipal, or insurance requirements, or with national safety codes and constitute no warranty. Any use of these data and information must be determined by the user to be in accordance with applicable federal, state, and local regulations.

All materials and mixtures may present unknown hazards and should be used with caution. Since Abraxis, Inc. cannot control the methods, volumes, or conditions of use of this product, Abraxis, Inc. shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material. This product is sold for research use only. It is not for any human or animal therapeutic or clinical diagnostic use.

Date this SDS was prepared: 5/24/2016

Version: 2

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