Importance of Histamine Determination

Histamine is the agent that causes scombroid poisoning, a foodborne chemical intoxication. Scombrosis is among the top three seafood illness reported in the Unites States. Many instances of histamine poisoning, especially in recreational-caught fish, go unreported to health officials. Large amounts of histamine can be formed in these fish if they are poorly handled and not stored at proper temperature.

The symptoms of histamine poisoning can include dizziness, headache, facial swelling and flushing, nausea, abdominal cramping, diarrhea, and difficulty in swallowing. Most victims recover within 24 hours especially with the aid of antihistamines. However, individuals sensitive to histamine can suffer for longer periods of time.

Fish contain relatively large amounts of the amino acid histidine in their muscle. After death, histamine forming bacteria that occurs naturally in fish (or added during handling) can transform the histidine to histamine by carboxylation. Therefore, histamine formed in foods is the result of the growth of bacteria that possess the enzyme histidine decarboxylase. Histamine is heat stable and survives thermal processing. The quality of fish, commercial fishmeal and other related products are directly related to the histamine content of these products.

In the USA, Histamine is regulated by the FDA in accordance to the implementation of the HACCP principles in the seafood industry. The guidelines for histamine in fish, and fishery products is 5 mg/100 g (50 ppm).

The Abraxis Histamine ELISA Kit provides a system screening system for histamine in fish and fish products.

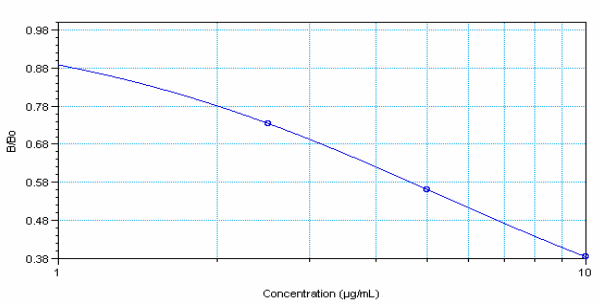
Performance Data

Test sensitivity: The detection limit for this assay is 0.8 ppm (µg/mL)

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

Selectivity: Other compounds tested at the stated levels were found to give results not greater than 1.0 µg/mL of histamine.

Compound	Conc. (µg/mL)	Inhibition (%)
Histidine	1000	< 5
Serotonin	1000	<5
Spermidine	500	<10
Putrescine	500	<10
Spermine	500	<10
Tyramine	500	<10
Cadaverine	500	<10



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 LLC makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

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

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ABRAXIS

Histamine ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Histamine in Fish Samples

Product No. 515168

1. General Description

The Abraxis Histamine ELISA is an immunoassay for the quantitative and sensitive detection of Histamine in fish extracts. If necessary, positive samples can be confirmed by HPLC, or other conventional methods.

2. Safety Instructions

The stop solution contains diluted sodium hydroxide. Avoid contact of the stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Histamine ELISA should be stored in the refrigerator (4–8°C). Solutions should be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA that allows the detection of Histamine. It is based on the recognition of Histamine by specific antibodies. Histamine, when present in a sample, and a Histamine-AP analogue compete for the binding sites of rabbit anti-Histamine antibodies that have been immobilized onto the microtiter wells. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the yellow color is inversely proportional to the concentration of the Histamine present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the calibration curve constructed with each run.

5. Limitations of the Histamine ELISA, Possible Test Interference

Many 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 samples, test interferences caused by matrix effects can not be completely excluded. Mistakes in handling the test also can 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 outside temperatures (lower than 10°C or higher than 35°C) during the test performance. The assay procedure should be performed away from direct sun light.

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

A. Materials Provided

1. Microtiter plate coated with anti-Histamine antibody. Twelve strips of 8 detachable wells.
2. Calibrator 0 (Negative Control), containing 0 µg/mL Histamine, 1.2 mL.
3. Calibrators (3), containing Histamine at the following concentrations: 2.5, 5.0, 10.0 µg/mL (ppm), 0.9 mL.
4. Histamine-Alkaline Phosphatase (AP) Enzyme Conjugate, 10.5 mL
5. Extract Solution/Sample Diluent (10X), 100 mL. Use to extract samples and to dilute samples with concentration above 100 µg/mL (ppm).
6. Wash Solution 10X Concentrate, 15 mL
7. Substrate/Color Solution (pNPP), 10.5 mL
8. Stop Solution (3 N NaOH), 5.5 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the calibrators and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the calibrator solutions and the samples on the entire microtiter plate. Please only use the reagents and calibrators from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The calibrators, control, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash solution is a 10X concentrated solution and needs to be diluted with deionized water. In a 0.25 L container dilute the 10X solution 1:10 (i.e. 15 mL of the 10X wash solution plus 135 mL of deionized water). The diluted solution is used to wash the microtiter wells.
5. The Extract/Sample Diluent solution is a 10X concentrated solution and needs to be diluted with deionized water. In a 1 L container dilute the 10X solution 1:10 (i.e. 100 mL of the 10X wash solution plus 900 mL of deionized water). The diluted solution is used to extract samples and to dilute extracts if necessary.

C. Assay Procedure

1. Add 50 µL of the calibrator solutions, negative control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 100 µL of the enzyme conjugate 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 for about 30 seconds. Be careful not to spill contents. Incubate the strips for forty (40) minutes at room temperature.
3. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times using the 1X washing buffer solution. Please use at least a volume of 250 µL 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. Alternatively a squeeze bottle may be used to fill the wells between each wash.
4. Add 100 µL of substrate/color solution to the wells using a multi- channel pipette or a stepping pipette. The strips are incubated for 20 minutes at room temperature. Protect the strips from sunlight.
5. Add 50 µL of stop solution to the wells in the same sequence as for the substrate/color solution using a multi- channel pipette or a stepping pipette.
6. Read the absorbance at 405 nm using a microplate ELISA photometer within 15 minutes after stopping the reaction.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the calibrators. Calculate the $\%B/B_0$ for each calibrator by dividing the mean absorbance value for each calibrator by the Zero Calibrator (Calibrator 0) mean absorbance. Construct a calibrator curve by plotting the $\%B/B_0$ for each calibrator on a vertical linear (y) axis versus the corresponding Histamine concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of Histamine by interpolation using the calibrator curve.

The concentrations of the samples are determined using the constructed calibration curve (do not use a previously stored curve). Samples showing a lower concentration than the lowest calibrator (2.5 ppm) of Histamine are considered to be negative. Samples showing a higher concentration than the highest calibrator 10.0 ppm must be diluted to obtain more accurate results.

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

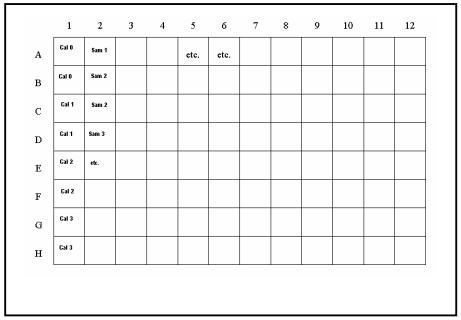
1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wavelength 405 nm)
6. 50 mL plastic extract tubes with caps
7. Overhead tube rotator or equivalent

F. Working Scheme

The microtiter plate consists of 12 strips of 8, which can be used individually. The calibrators must be run with each test. Never use the values of calibrators which have been determined in a test performed previously.

Calibrators : 0, 2.5, 5, 10 ppm

Sam1, Sam2, Sam3, etc.: Samples



G. Preparation and Extraction of Fish Samples

1. Weigh a 2.0 gm of a representative sample into a 50 mL plastic extraction tube.
2. Add 20 mL of the 1X Extraction solution, close tightly.
3. Shake the tube continuosly for one minute, set aside at room temperature for 5 minutes. Shake again for 20 minutes.
4. Allow contents to settle for 3-5 minutes.
5. Sample is ready to test in the ELISA. Without disturbing the tube, use the supernatant (top portion) for assay. If necessary, dilute the supernatant with 1X Sample Diluent solution to 1:10 and 1:50 before testing.

The Histamine concentration contained in the samples is determined by multiplying the concentration of the extract by the dilution factor used. Highly contaminated samples outside the range of the curve should be diluted further and re-analyzed.

Sensitivity

The Abraxis Histamine ELISA has an estimated minimum detectable concentration, based on 90% B/Bo of 0.8 µg/mL (ppm).

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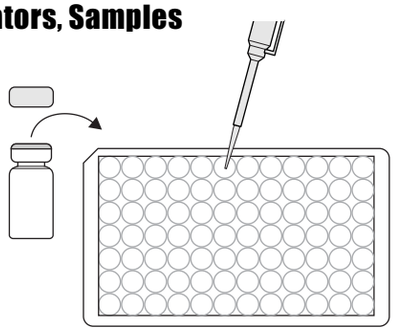
Histamine Plate, Detailed ELISA Procedure

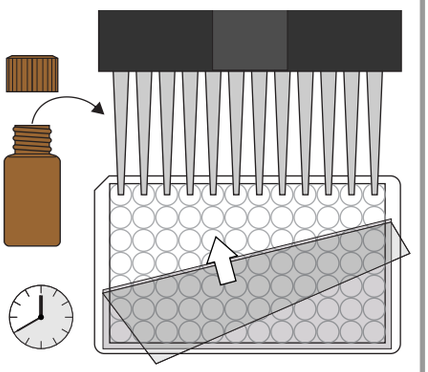
1. Addition of Calibrators, Samples

Add 50 ul of the calibrator solutions, negative control, or samples to the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



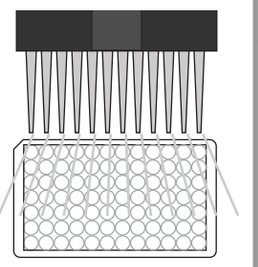
2. Addition of Conjugate Solution

Add 100 uL of the enzyme conjugate 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 40 min at room temperature.

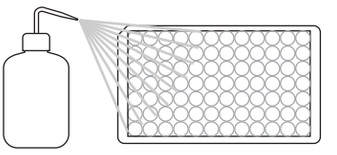


3. Washing of Plates

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 a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.

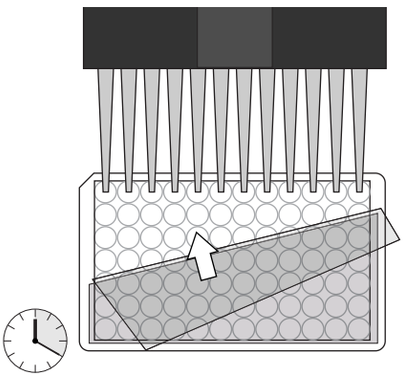


OR



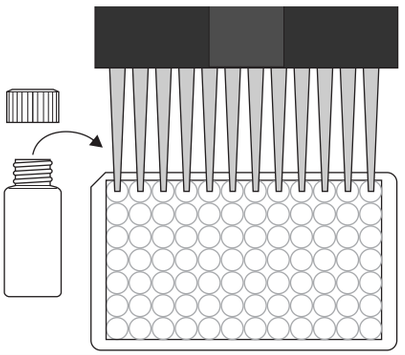
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20 min at room temperature.



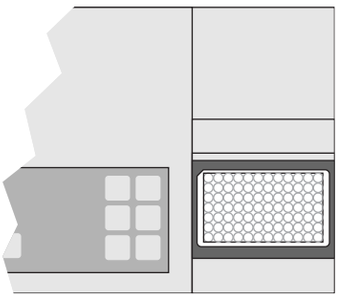
5. Addition of Stopping Solution

Add 50 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.



6. Measurement of Color

Read the absorbance at 405 nm using a microplate ELISA reader. 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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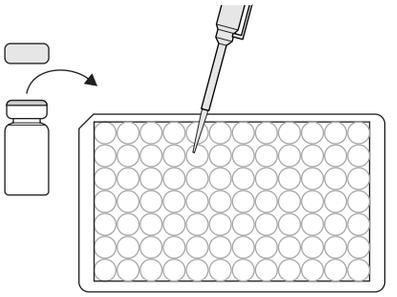
Histamine Plate, Concise ELISA Procedure

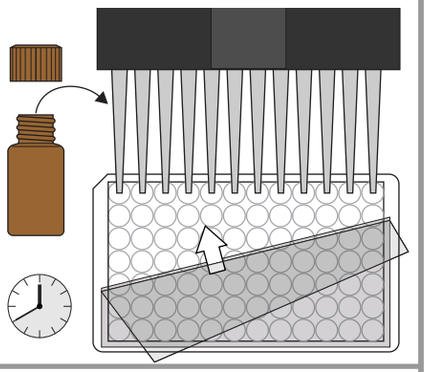
1. Addition of Calibrators, Samples

Add 50 ul of the calibrator solutions, negative control, or samples.



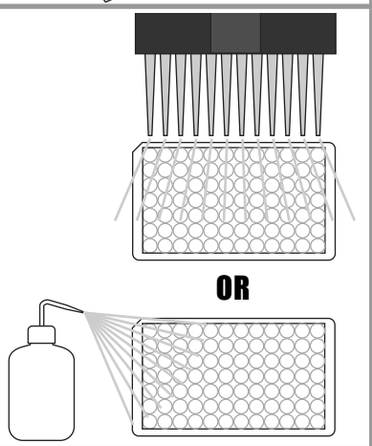
2. Addition of Conjugate Solution

Add 100 uL of the enzyme conjugate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 40 min at room temperature.



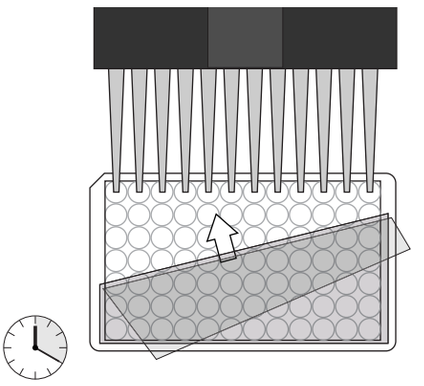
3. Washing of Plates

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



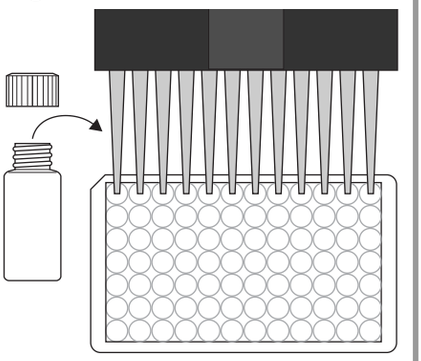
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 20 minutes at room temperature and away from direct sunlight.



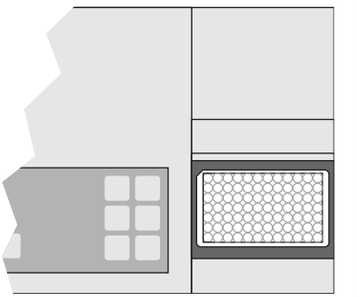
5. Addition of Stopping Solution

Add 50 uL of stop solution.



6. Measurement of Color

Measure color at 405 nm. Calculate results.



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

Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: Histamine ELISA Plate Kit
Product Code: 515168
1.2 Identified Use: Determination of Histamine 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: 5/20/2016
Version: 2
Changes from previous version: Abraxis, LLC changed to Abraxis, Inc.