Importance of Diuron Determination

One of the most frequently used herbicides is diuron, it belongs to the class of phenylurea herbicides. They are applied for weed control e.g. on railway lines, roads, parking lots or industrial areas as well as for algae control in fish ponds. It is desirable to check water samples for possible residues of diuron as this herbicide may frequently occur in water and soil.

The diuron ELISA allows the determination of 40 samples in duplicates. Only a few mL of sample are required. The test can be performed in less than 1 hour.

Performance data

Test sensitivity: The detection limit for diuron is 0.03 µg/L (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B_0) is at 0.25 µg/L. Determinations close to the middle of the tests yield the most accurate results.

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

Selectivity: The ELISA for diuron recognizes also linuron, chlorbromuron and neburon (CR: >10%).

Cross-reactivities:

diuron	100% (per definition)
neburon	1250 %
chlorbromuron	62.5 %
linuron	25 %
chlortoluron	7.8 %
propanil	4.8 %
monuron	<1 %
monolinuron	<1 %
fenuron	<1 %
bromuron	<1 %
isoproturon	<1 %
propham	<1 %

Cross-reactivities with herbicides different from phenylureas have not been observed.

Samples: Drinking water, ground water and surface water samples were tested for matrix effects in the ELISA. No matrix effects were determined.

Recovery: Spiking of samples with different concentrations of diuron (0.05-3 µg/L) yielded a recovery of 80-110%.

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

Diuron ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Diuron in Water Samples

Product No. 520001

1. General Description

The diuron ELISA is an immunoassay for the sensitive determination of diuron, a phenylurea herbicide. This test is suitable for the determination of diuron in water samples. A previous sample preparation is not required. If required, positive samples can be analyzed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions of the test kit contain the herbicide diuron. In addition to this the substrate solution contains tetramethylbenzidine and the stop solution sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If this reagent comes in contact with skin wash with water.

3. Storage and Stability

The diuron ELISA has to be stored in the refrigerator (4–8°C). The solutions have to be adjusted to room temperature (20-25°C) before use of the test kit. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is based on the recognition of diuron by specific antibodies. Diuron present in the sample and a phenylurea-enzyme conjugate compete for the binding sites of the antibodies immobilized on the plate. After a washing step and addition of the substrate solution a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of diuron present in the sample. The color reaction is stopped after 20 min and the color is evaluated using an ELISA reader.

5. Limitations of the Diuron ELISA, Possible Test Interference

Water samples may contain a number of various ingredients. Due to the high variability of possible ingredients, test interference caused by matrix effects cannot 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 sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures during the test performance (lower than 15°C or higher than 30°C).

Life Technologies™
Your Molecular & Cell Technology Partner

Working Instructions

A. Test Preparation

Micro-pipetting equipment and the matching pipet tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipet or a stepping pipet for adding the enzyme tracer, the substrate solution and the stop solution in order to equalize the incubation periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted to each other.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. The washing buffer concentrate (5x concentrated) has to be diluted 5-fold to the amount required with distilled H_20 (i.e. 100 mL of wash buffer 5X and 400 mL of DI water). The number of microtiter plate strips required is removed from the aluminum foil. The remaining strips are stored back in the aluminum foil and closed again using the white plastic clip. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, positive and negative controls, enzyme tracer, substrate and stop solution are ready to use and do not require any further dilutions.
4. The stop solution has to be handled with care as it contains diluted sulfuric acid.

B. Assay Procedure

1. Add 25 µL of the assay buffer into each individual well using a multi-channel or stepping pipet.
2. Add 50 µL of the standard solutions, the controls or the samples into the wells of the individual test strips according to the working scheme given. We recommend using duplicates or triplicates.
3. Add 50 µL of enzyme conjugate solutions to the individual wells successively using a multi-channel pipet or a stepping pipet.
4. Incubate the strips for 30 min at room temperature (if possible use an orbital shaker).
5. Wash the strips three times using the washing buffer solution. Please use at least a volume of 300 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 stock of paper.
6. Add 100 µL of substrate solution to the wells. The strips are incubated for 25-30 min at room temperature in the darkness, if possible on a shaker. Protect the strips from light.
7. Add 50 µL of stop solutions to the wells in the same sequence as for the substrate solution.
8. Read results at 450 nm using an ELISA photometer.

C. Evaluation

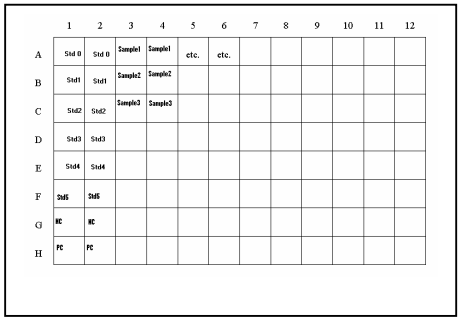
The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (Logit/Log or 4-Parameter). For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for the Zero Standard (Standard 0). Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding diuron concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of diuron by interpolation using the standard curve.
The concentrations of the samples are determined using this standard curve. Samples showing a lower concentrations of diuron compared to standard 1 (0.03 µg/L) are considered as negative. Samples showing a higher concentration than standard 5 (3 µg/L) must be diluted further to obtain more accurate results. The concentration of the negative and positive controls should be in the range given in the test instructions (±20%).

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

1. Micro-pipets with disposable plastic tips (10-100 µL, 100-1000 µL)
2. Multi-channel pipet (10-200 µL) or stepper pipet with plastic tips (10-200 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates

E. Working Scheme

The microtiter plate consists of 12 X 8 strips, which can be used individually for the test. The standards have to be applied in each test. Do not use the values of standards, which have been determined in a test performed previously.

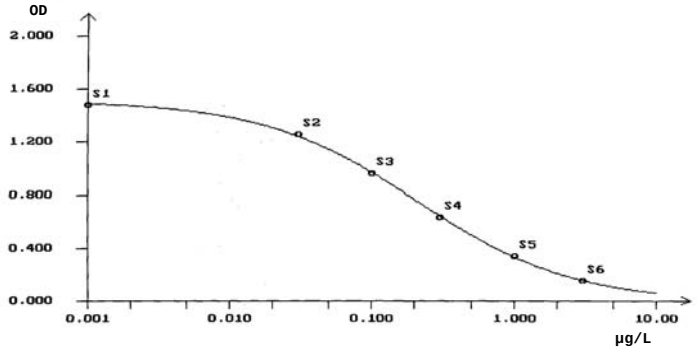


St0-St5: Standards
(0,0.03, 0.10, 0.3, 1.0, 3.0, ug/L)
NC (Negative Control): <0.03 µg/L
PC (Positive Control): 0.30 µg/L

Sa1, Sa2, Sa3, etc.: Samples

F. Standard Curve

(These values are used for demonstration purposes; do not use these values for your determinations)



G. References

(1) P. Schneider, M.H. Goodrow, S.J. Gee, B.D. Hammock, A highly sensitive and rapid ELISA for the arylurea herbicides diuron, monuron and linuron. J. Agric. Food Chem. 42, 1994, 413-422.

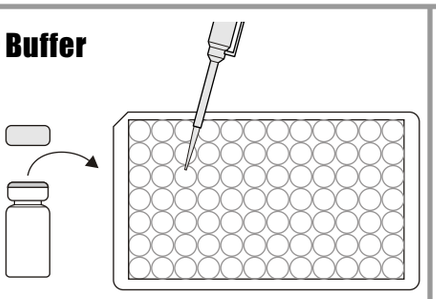
(2) B. Hock, T. Giersch, A. Dankwardt, K. Kramer, S. Pullen, Toxicity Assessment and On-line monitoring: Immunoassays, Environ. Toxicol. Water Qual. 9, 1994, 243-262.

Life Technologies™
Your Molecular & Cell Technology Partner

Diuron Plate, Detailed ELISA Procedure

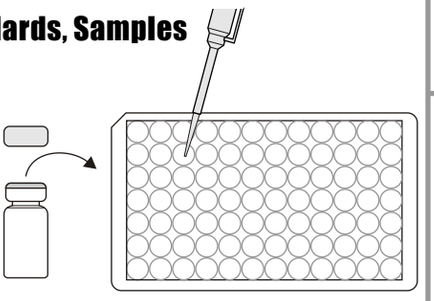
1. Addition of Assay Buffer

Add 25 ul of the Assay Buffer into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



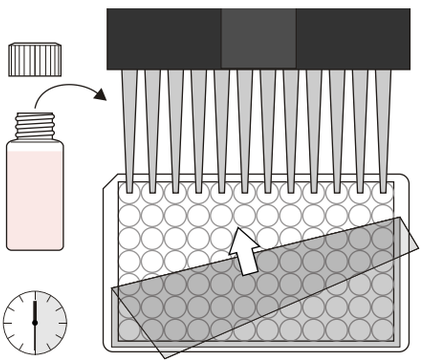
2. Addition of Standards, Samples

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



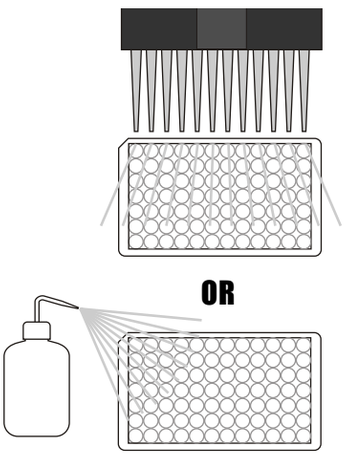
3. Addition of Enyzme Conjugate

Add 50 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 for 30 seconds. Be careful not to spill contents. Incubate for 30 minutes at room temperature.



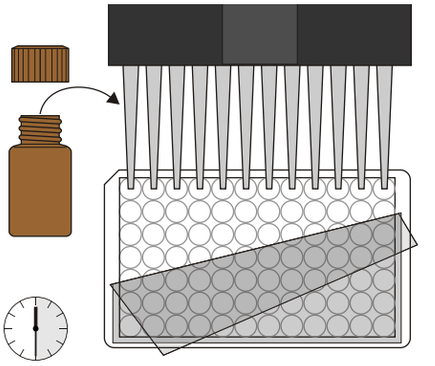
4. Washing of Plates

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 1X washing buffer solution. Please use at least a volume of 300 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.



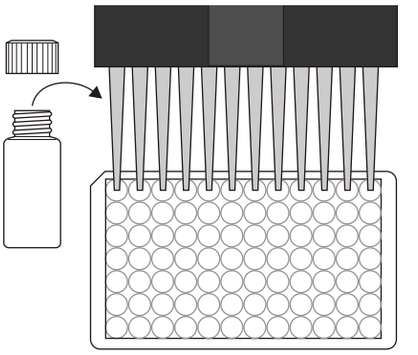
5. Addition of Substrate/Color Solution

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 25-30 min at room temperature.



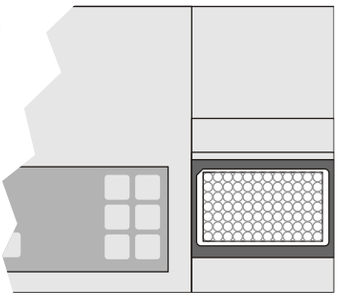
6. Addition of Stopping Solution

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.



7. Measurement of Color

Read the absorbance at 450 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

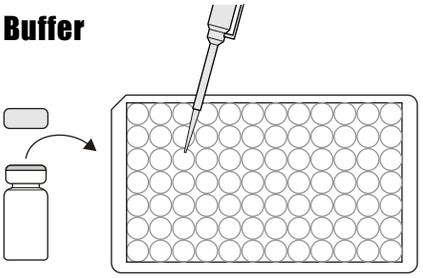
Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

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

Diuron Plate, Concise ELISA Procedure

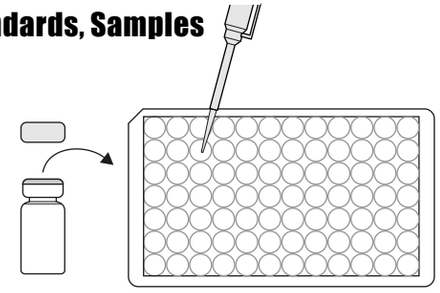
1. Addition of Assay Buffer

Add 25 uL of the Assay Buffer.



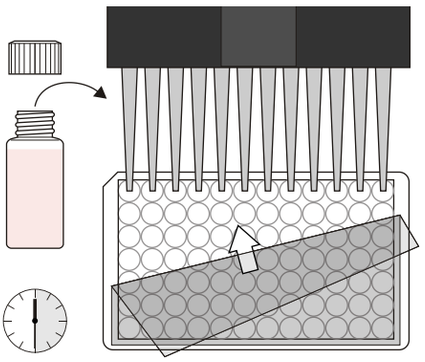
2. Addition of Standards, Samples

Add 50 uL of standard solutions, control or samples.



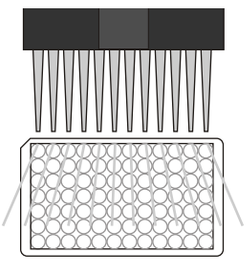
3. Addition of Enyzme Conjugate

Add 50 uL of enzyme conjugate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.

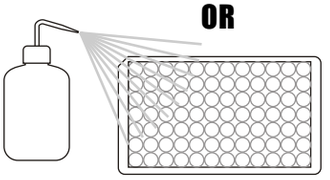


4. Washing of Plates

Wash the plates three times with 300 uL of 1X washing buffer.

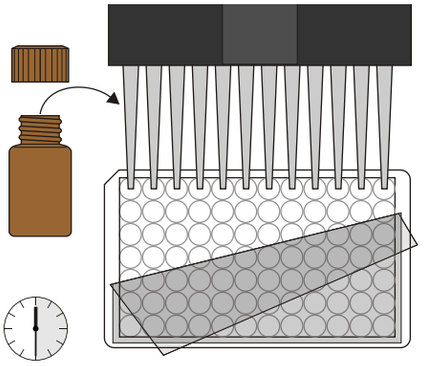


OR



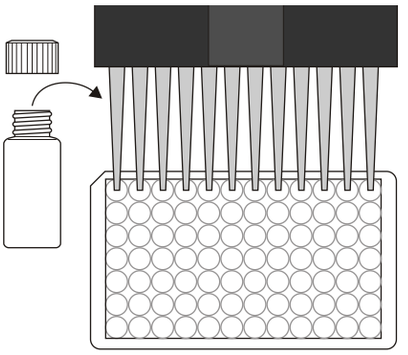
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 25-30 minutes at room temperature and away from direct sunlight.



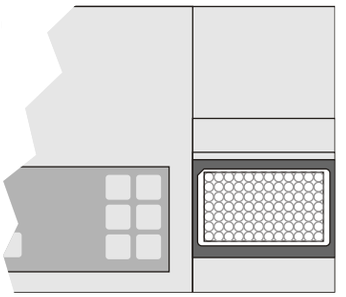
6. Addition of Stopping Solution

Add 50 uL of stop solution.



7. Measurement of Color

Measure color at 450 nm. 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
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: Diuron ELISA Plate Kit
Product Code: 520001
1.2 Identified Use: Determination of Diuron 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
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.