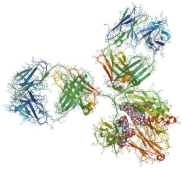

Ludger

Product Guide for LudgerTag™ 2-AA (2-aminobenzoic acid) Glycan Labeling Kit containing 2-picoline borane

Part of the Ludger-Velocity™ Fast Glycan Analysis Range.

(Ludger Product Code: LT-KAA-VP24)

Ludger Document # LT-KAA-VP24-Guide-v2.0

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Note: The use of 2-picoline borane in labeling reactions is exclusively licensed to Ludger Ltd

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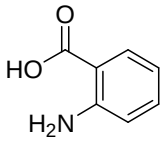
Specifications for LT-KAA-VP24

Application For labeling of free glycans with 2-aminobenzoic acid acid (2-AA).

Description The kit contains reagents for the conjugation of dye to the free reducing end of the glycan
by a reductive amination reaction.

Dye Properties Mass = 137. Fluorescence, λ_{ex} (glycan-dye conjugate) = 250 nm, λ_{em} = 425 nm. For maximum sensitivity of detection we recommend an excitation wavelength of 250 nm.



Number of Samples 12 separate analytical samples per set of labeling reagents (24 samples in total per kit)

Amount of Sample From 25 pmol up to 25 nmol glycans per sample.

Suitable Samples Any purified glycans with free reducing termini can be labeled.

Structural Integrity No detectable (< 2 mole per cent) loss of sialic acid, fucose, sulfate, or phosphate.

Labeling Selectivity Essentially stoichiometric labeling.

Storage: Store at room temperature in the dark. Protect from sources of heat, light, and moisture. The reagents are stable for at least two years as supplied.

Shipping: The product can be shipped at ambient temperature.

Handling: Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate.
All steps involving labeling reagents must be performed in a dry environment with dry glassware and plasticware. Once individual vials of reagents are opened, their contents should be used immediately and excess then discarded according to local safety rules.

Safety: **For research use only. Not for human or drug use**
Please read the Material Safety Data Sheets (MSDS's) for all chemicals used. All processes involving labeling reagents should be performed using appropriate personal safety protection - eyeglasses, chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard.

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Kit Contents

Each kit contains two labelling reaction sets. Each labeling reaction set consists of one vial of each of the following:

Cat. #	Item	Quantity
LT-2AA-03	2-AA Dye (2-aminobenzoic acid)	7.5 mg
LT-PB-01	2PB reductant (2-picoline borane)	16.5 mg
LT-ACETIC-DMSO-01	30% acetic acid in DMSO	500 µl

Additional Reagents and Equipment Required

- Milli Q water or similar
- Heating block, oven, or similar dry heater (a water bath cannot be used) set at 65°C
- Centrifugal evaporator (e.g. Savant, Heto, or similar)
- Reaction vials (e.g. polypropylene microcentrifuge vials)
- Note: Further reagents are required if doing the optional post-labeling sample cleanup (see Section on Sample Cleanup)

Time Line for Labeling

The LudgerTagÊ labeling procedure takes 2 hours with just 1 hour for the actual labelling incubation.

Procedure	Time	Elapsed Time (hours)
Transfer samples to reaction tube and dry	30 min	0.5
Add water to samples	15 min	0.75
Make up and add labeling reagent	15 min	1
Incubate samples with reagent	**1 hour**	2

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Labeling Method

1 Purify the glycans

If necessary, remove non-carbohydrate contaminants from the samples (Ludger product LC-EB10-A6).

2 Transfer sample to reaction vial

The kit is designed to label up to 25 nmols of glycans per reaction. With a single pure glycan as little as 5 picomoles per reaction can be labeled and detected in subsequent HPLC analysis. Suitable reaction vials include small polypropylene microcentrifuge tubes and tubes for PCR work.

3 Dry the samples and resuspend in 10 µL of water

Dry down the samples if the volume of the sample exceeds 10 µL.

If the samples need to be dried down then this should be done using a centrifugal evaporator. If this is not possible then freeze drying (lyophilization) can be used with caution (in particular, ensure that the sample dries to a small, compact mass at the very bottom of the vial). Do not subject samples to high temperatures (>28°C) or extremes of pH as these conditions will result in acid catalysed loss of sialic acids (high temperatures, low pH) or epimerization of the glycan reducing terminus (at high pH).

Once the samples are dry then redissolve the glycans in 10 µL of water.

4 Prepare the labelling reagent

Add 150 µl of kit component LT-ACETIC-DMSO-01 (30% acetic acid in DMSO) to a vial of dye (LT-2AA-03) and mix by pipette action until the dye is dissolved. Sometimes heat (30-60°C) is required to help dissolve the dye.

Transfer the 150 µL of dissolved dye solution to a vial of reductant (LT-PB-01) and mix by pipette action until the reductant is dissolved. Sometimes heat (30-60°C) is required to help dissolve the reductant.

5 Add labeling reagent to samples

Add 10 µl of labeling reagent to each glycan sample, cap the microtube, mix thoroughly, and then gently tap to ensure the labeling solution is at the bottom of the vial.

6 Incubate

Place the reaction vials in a heating block, sand tray, or dry oven set at 65°C and incubate for 1 hour.

The samples must be completely dissolved in the labeling solution for efficient labeling. To encourage complete dissolution the samples can be vortexed 30 minutes after the start of the 65°C incubation then the incubation continued.

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7 Centrifuge and cool

After the incubation period remove the samples, centrifuge the microtubes briefly, and then allow them to cool completely to room temperature.

LudgerClean™ S or T1 Post-Labeling Sample Cleanup

Post-labeling sample cleanup (to remove excess dye and other labeling reagents) is necessary for certain applications - e.g. subsequent analysis by HPLC. Such cleanup can be achieved using LudgerCleanÊ S cartridges (Cat # LC-S-Ax, where x denotes the number of cartridges in the kit) or 96 well compatible LudgerCleanÊ T1 cartridges (Cat # LC-T1-Ax, where x denotes the number of cartridges in the kit) both using the standard protocol included with the kits.

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Analysis of LudgerTag™ 2-AA-Labeled Glycans

LudgerTagÊ 2-AA labeled glycans may be studied by a number of different analytical methods including HPLC, gel electrophoresis, and mass spectrometry.

HPLC Analysis

LudgerTagÊ 2-AA labeled glycan mixtures may be separated and analysed by a variety of HPLC (high pressure liquid chromatography) methods including LudgerSep™ HPLC:

Type of Analysis	Column
Separation of charged and neutral glycans	LudgerSepÊ C
Profile analysis of neutral and charged glycans	LudgerSepÊ N
Separation of neutral glycans	LudgerSepÊ R

The LudgerSepÊ N column is an especially powerful tool for the purification and analysis of LudgerTagÊ labeled oligosaccharides from complex glycan mixtures. Please contact us for advice regarding your particular application.

Enzymatic Analysis

High purity, sequencing grade enzymes (e.g. exoglycosidases) suitable for structural analysis of both N- and O-linked LudgerTagÊ labeled glycans are available from a number of companies.

When selecting glycosidases be especially careful to choose those with formulations that are compatible with your particular application. For example, some enzymes and enzyme buffers have components that interfere with certain types of analysis. Please call us for guidance in selecting enzymes and reaction conditions for your work.

Mass Spectrometry and Electrophoresis

LudgerTagÊ labeled glycans may also be analysed by mass spectrometry, electrophoresis, and various types of spectroscopy. Please call us for advice on the analysis conditions most suitable for your intended analyses.

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The Reductive Amination Reaction

The labeling reaction involves a two step process (see Figure 1):

1. **Schiff's base formation.**
   This requires a glycan with a free reducing terminus which is equilibrium between the ring closed (cyclic) and ring open (acyclic) forms. The primary amino group of the dye performs a nucleophilic attack on the carbonyl carbon of the acyclic reducing terminal residue to form a partially stable Schiff's base.

2. **Reduction of the Schiff's base.**
   The Schiff's base imine group is chemically reduced to give a stable labeled glycan.

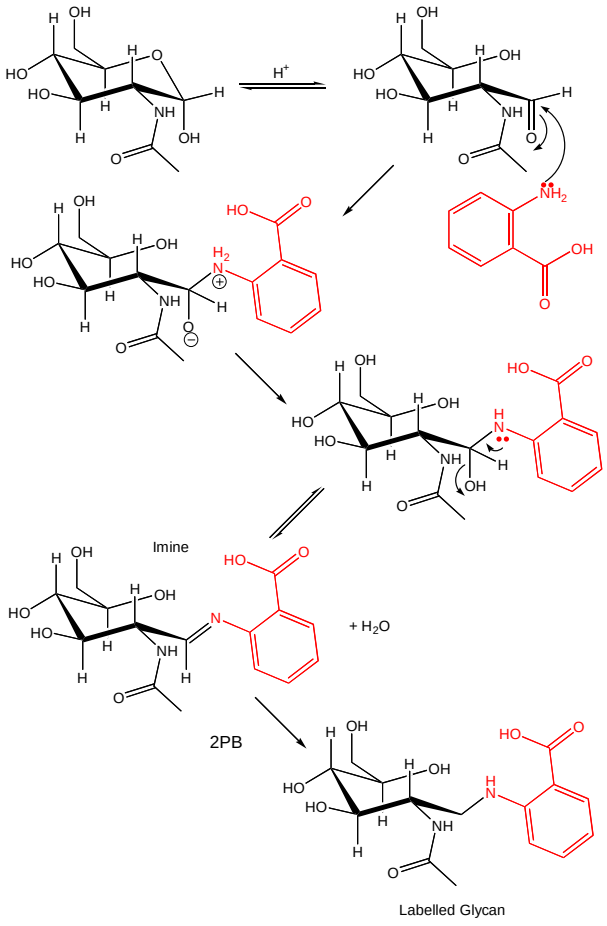

Figure 1: Labeling of a glycan with 2-aminobenzamide acid (2-AA) by reductive amination.

Warranties and Liabilities

Ludger warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse Ludger will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and Ludger makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.
Ludger shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

Document Revision Number

Document # LT-KAA-VP24-Guide-v2.0

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Appendix 1: Troubleshooting Guide

The glycan sample to be labeled, whether a purified glycan or a glycan mixture, must contain a free reducing terminus, be particle and salt-free, and be presented in a volatile solvent system (preferably pure water).

The following may interfere with the labeling reaction and must be removed from the glycan samples prior to LudgerTagÊ labeling:

- Non-volatile solvents
- Non-volatile salts, in particular transition metal ions
- Detergents
- Dyes and stains such as Coomassie Blue

A range of LudgerCleanÊ kits for cleaning glycan samples prior to LudgerTagÊ labeling is available from Ludger.

The LudgerTagÊ labeling protocol is an efficient, robust method. If problems do arise they can normally be corrected without difficulty. The following is a guide to the most likely problems, possible causes, and solutions.

Poor Incorporation of Dye / Low Labeling Yield

Water was not added to the glycans prior to adding the labelling reagent

Please ensure that 10 µL of water is added to the glycans during the labelling step. The water can be used to solubilise the glycans prior to adding the labelling reagent or added after the labelling reagent. Water is necessary in order to maximise the labelling efficiency.

The labeling temperature was incorrect.

Please ensure that the oven or heating block is equilibrated to the incubation temperature and that the reaction tube is subjected to this temperature for the entire labeling period.

The sample was incompletely solubilised.

The glycans must be completely dissolved in the labeling mixture for maximum labeling efficiency. Please ensure that the sample is thoroughly mixed with the labeling reagent prior to the incubation and, as a precaution, carefully mix the samples 15 minutes after the start of the incubation.

The sample contained contaminants that interfered with the labeling.

Please ensure that the glycans are adequately purified before labeling (see protocol step 1 and the LudgerCleanÊ Glycan Cleanup Guide).

The labeling solution was inactive.

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Please make up the labeling solution immediately before use - the reagents will lose activity within a few hours of mixing.

There was less starting glycan than was originally estimated.

The glycans did not contain a free reducing terminus.

The 2-AA dye conjugates to the glycan via the aldehyde group of the free reducing terminus. Alditols and glycans already conjugated via their reducing terminus (e.g. glycopeptides, glycolipids, and previously labeled glycans) do not contain a free reducing terminus and so cannot conjugate to the dye.

The glycans were lost during the post-labeling cleanup.

Please ensure that the removal of excess labeling reagents is performed as specified in the cleanup protocol and that the wash reagents are correctly made.

The Labeled Samples Contain Fluorescent Non-Carbohydrate Material

The original glycans contained aldehyde-bearing contaminants.

Please ensure that the glycans are adequately purified before labeling (see protocol step 1 and the LudgerClean Glycan Cleanup Guide).

The post-labeling cleanup step did not work correctly.

Please ensure that the removal of excess labeling reagents is performed as specified in the post-labeling cleanup protocol and that the wash reagents are correctly made.

Selective Loss of Smaller Glycans

The cleanup cartridge was not primed correctly.

Please ensure the cartridge is primed correctly and that the cartridge bed is still wet with acetonitrile when the sample is applied to the disc.

Incorrect wash reagents were used during the post-labeling cleanup.

Please ensure that the wash reagents are correctly prepared.

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Selective Loss of Larger Glycans

The sample was incompletely solubilised.

The glycans must be completely dissolved in the labeling mixture for maximum labeling efficiency. Larger glycans tend to be less soluble in the labeling mixture than small sugars. Please ensure that the sample is thoroughly mixed with the labeling reagent prior to the incubation and, as a precaution, carefully mix the samples 15 minutes after the start of the incubation.

Desialylation of the Glycans

The sample was subjected to acidic conditions in aqueous solutions at elevated temperatures

Avoid prolonged periods of exposure of sialylated glycan samples in aqueous solutions to conditions of low pH and elevated temperatures. Keep the incubation time of the labelling reaction to 1h as desialylation increases with 2 or 3h incubation times.

The samples were not cleaned up correctly after labeling

Make sure that samples undergo the post-labeling cleanup immediately after the reductive amination reaction and that the post-labeling drying and cleanup procedure is conducted reasonably quickly.

Labeled samples that have **not** undergone drying and subsequent cleanup will be prone to acid catalyzed de-sialylation.

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SAFETY DATA SHEET

Version: 1.0 Date written: 13th November 2012

SECTION 1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY / UNDERTAKING

Product Name	**Acetic Acid / dimethyl sulfoxide solution**
Product Catalogue Name	**LT-ACETIC-DMSO-01**
Company:	Ludger Ltd Culham Science Centre Abingdon Oxford OX14 3EB
Telephone:	01865 408554
Emergency Telephone:	01865 408554
Email:	info@ludger.com

SECTION 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture
Classification according to Regulation (EC) No. 1272/2008 [EU-GHS-CLP]
Flammable liquids (Category 3)
Skin corrosion (Category 1A)

2.2 Label elements

Signal Word: Danger

Hazard Statement(s)

H226	Flammable liquid and vapour
H314	Causes severe skin burns and eye damage.

Precautionary Statement(s)

P280	Wear proactive gloves/ protective clothing/ eye protection/ face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and safe to do so. Continue rinsing.
P310	Immediately call a POISON CENTRE or doctor/ physician.

2.3 Other hazard information:
None

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3. 1 Substances

Synonyms: DMSO, methyl sulfoxide, dimethyl sulfoxide
Glacial acetic acid

Formula: DMSO: C_2H_6OS
Acetic Acid: $C_2H_4O_2$

Molecular Weight: DMSO: 78.13 g/mol
Acetic Acid: 60.05 g/mol

Component		Concentration
Name	Dimethyl Sulfoxide	70%
CAS-No.	67-68-5	
EC-No.	200-664-3	
Name	Acetic Acid	30%
CAS-No.	64-19-7	
EC-No.	200-580-7	
Index-No.	607-002-00-6	

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures

General Advice
Consult a physician if exposure causes ill effects and if in any doubt. Show this safety data sheet to the physician/ first responder in attendance.

If Ingested
Do NOT induce vomiting. Rinse mouth well with water. Never give anything by mouth to an unconscious person.

If skin is exposed
Remove all contaminated clothing immediately; wash the area well with plenty of soap and water.

If eyes are exposed
Flush eyes with plenty of water/ eye wash solution for at least 15 minutes, if present and safe to do so, remove contact lenses and continue rinsing.

If inhaled
Move effect person to fresh air. If not breathing give artificial respiration.

4.2 Most important symptoms and effects, both acute and delayed
Nausea, Fatigue and Headache. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

4.3 Indication of immediate medical attention and special treatment needed
No data available.

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SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media
Small fires: Use extinguishing media such as ñalcoholòfoam, dry chemical or carbon dioxide.
Large fires: Use extinguishing media such as water, from a far away distance as possible. Use very large quantities of water as mist or spray to flood the fire and the combustible material. Cool all affected containers with large quantities of water.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, Sulphur oxides

5.3 Advice for fire fighters
Wear self contained breathing apparatus fir fire fighting if necessary, to spray cool water on any unopened containers near the fire.

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Avoid breathing vapours, gas or mist. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

6.2 Environmental Precautions
Prevent further leakage or spillage if safe to do so, e.g. with spill mats. Do not let the product enter drains.

6.3 Methods and material for containment and cleaning up
Contain the spillage and put the collected material into a suitable container with a secure lid. Wash the area well, do not let run off into the drains, collect as waste.

6.4 Reference to other sections
See section 13 for disposal of waste material(s).

SECTION 7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid inhalation of vapour or mist. Keep away from sources of ignition- No smoking.
Take measures to prevent the build up of electrostatic charge.

7.2 Conditions for safe storage, including any incompatibilities
Store in a cool place. Keep container closed in a dry well ventilated place.

7.3 Specific end uses
No data available

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters.

ACETIC ACID

CAS-No.	Value	Control Parameters	Update	Basis
64-19-7	TWA	10ppm 25mg/m3	1991-07-05	Europe. Commission Directive 91/322/EEC on establishing indicative limit on values.

Remarks	Indicative

DMSO contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls

Handle in accordance with good laboratory hygiene and safety practice. Wash hands before breaks and at the end of the day.

Personal Protective Equipment

Eye / face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

Skin protection

Handle with gloves, which should be inspected before use. Use proper glove removal technique (removal without the outside of the glove touching the skin) to avoid contact with the skin/chemical. Dispose of contaminated gloves as Laboratory waste in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Gloves should be of the standard that will stratify the specifications of EU directive 89/696/EEC and the standard EN 374 derived from it.

Body Protection

The type of protective clothing must be selected according to the amount of substance at the specific workplace being used. Impervious coats or laboratory coats.

Respiratory protection

Use substance in an operation fume hood/ outside venting extraction cupboard. Wear full face respirator if appropriate to use, must be tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Form: Liquid, clear Colour: Colourless
Odour	Strong
Odour threshold	No data available
pH	No data available
Freezing/Melting Point	No data available
Initial boiling point and boiling range	No data available
Flash Point	No data available
Evaporation rate	No data available
Flammability	No data available
Upper/lower flammability or explosive limits	No data available
Vapour Pressure, Pa at temperature degree C	No data available
Relative Density	No data available
Solubility in water and solvents	Completely miscible
Partition coefficient: n-octanol/water	No data available
Auto ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidising properties	No data available

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9.2 Other information
No data available

SECTION 10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available

10.2 Chemical stability
No data available

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to Avoid
Heat, flames and sparks

10.5 Incompatible materials
Acid chlorides, Phosphorus halides, Strong oxidizing agents and strong reducing agents, soluble carbonates and phosphates, hydroxides, metals, peroxides, permanganates, e.g. potassium permanganate, Amines and Alcohols.

10.6 Hazardous decomposition products
Other decomposition products ï No data available

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects
DMSO
Acute toxicity
LD50 Oral ï Rat ï 14,500mg/kg
LC50 Inhalation ï Rat ï 4h ï 40250ppm
LD50 Dermal ï Rabbit - > 5,000mg/kg

Acetic Acid
Acute toxicity
LD50 Oral ï Rat ï 3,310 mg/kg

LC50 Inhalation ï Mouse ï 1h - 5620ppm
Remarks: Sense Organs and Special Senses (Nose, Eyes, Ears and Taste): Eyes: Conjunctive irritation. Eyes: Other. Blood: Other changes.

LD50 Dermal ï Rabbit ï 1,112 mg/kg

DMSO
Skin corrosion/irritation
Skin ï Rabbit ï No skin irritation ï 4h

Acetic Acid
Skin corrosion/irritation
Skin ï Rabbit ï Mild skin irritation ï 24h

DMSO
Serious eye damage/irritation
Eyes ï Rabbit ï Mild eye irritation

Acetic Acid

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Serious eye damage/irritation
Eyes ï Rabbit ï Corrosive to eyes.

Respiratory or skin sensitisation
May cause sensitization by skin contact.

Germ cell mutagenicity
Genotoxicity in vitro ï Mouse ï lymphocyte
Cytogenetic analysis
Genotoxicity in vitro ï Mouse ï lymphocyte
Mutation in mammalian somatic cells

Genotoxicity in vivo ï Rat ï Intraperitoneal
Cytogenetic analysis

Genotoxicity in vivo - Mouse ï Intraperitoneal
DNA damage

Carcinogenicity
Carcinogenicity ï Rat ï Oral
Tumorigenic: Equivocal tumorigenic agent by RTECS criteria. Skin and Appendages: Others: Tumors.

Carcinogenicity ï Mouse ï Oral
Tumorigic: Equivocal tumorigenic agent by RTECS criteria. Lukaemia skin and appendages: Other: Tumors.

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

Reproductive toxicity
Reproductive toxicity ï Rat ï Intraperitoneal
Effects on fertility: Abortion

Reproductive toxicity ï Rat ï Intraperitoneal
Effects on fertility: Post ï implantation mortality (e.g. dead and/or resorbed implants per total number of implants).

Reproductive toxicity ï Rat ï Subcutaneous
Effects on fertility: Post ï implantation mortality (e.g. dead and/or resorbed implants per total number of implants). Effects on fertility: Litter size (e.g. # fetuses per litter; measured before birth).

Reproductive toxicity ï Mouse ï Oral
Effects on fertility: Pre-implantation mortality (e.g. reduction in number of implants per female; total number of implants per corpora lutea). Effects on Embryo or fetus: Fetoxicity (except death, e.g. stunted fetus). Specific developmental abnormalities: Musculoskeletal system.

Reproductive toxicity ï Mouse ï Intraperitoneal
Effects on embryo or fetus: Fetoxicity (except death, e.g. stunted fetus). Specific developmental abnormalities: Musculoskeletal system.

STOT-single exposure
No data available

STOT-repeated exposure
No data available

Aspiration hazard.

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No data available

Potential Health Hazards

Inhalation	Harmful if inhaled. Causes serious respiratory tract irritation.
Ingestion	Harmful if swallowed. Causes burns.
Skin	May be harmful if absorbed through skin. Causes skin burns.
Eyes	Causes eye irritation/ burns.
Aggravated Medical Condition	Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through the skin and may carry such materials into the body.

Signs and symptoms of exposure
Nausea, Fatigue, Headache. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

Additional Information
RTECS: PV6210000
RTECS: AF1225000

SECTION 12. ECOLOGICAL INFORMATION

12.1 Toxicity

DMSO

Toxicity to Fish	LC50-Pimephales promelas (fathead minnow) ï 34,000mg/l - 96h
	LC50-Oncorhynchus mykiss (rainbow trout) ï 34,000mg/l-96h
Toxicity to daphnia and other Aquatic invertebrates	EC50-Daphnia pulex (water fleas) ï 27,500mg/l
Toxicity to algae	EC50-Lepomis macrochirus (bluegill) - >400,000mg/l-96h

Acetic Acid

Toxicity to Fish	LC50 ï Leuciscus idus (Golden Orfe) ï 410.00mg/l ï 48h LC50 ï Cyprinus carpio (Carp) ï 49.00mg/l ï 48h LC50 ï Pimephales promelas (Fathead minnow) ï 79.00 - 88.00mg/l ï 96h LC50 ï Lepomis macrochirus ï 75mg/l ï 96h
Toxicity to Daphnia and other aquatic invertebrates.	EC50 ï Daphnia magna (Water flea) ï 65.00mg/l ï 48h

12.2 Persistence and degradability
Biodegradability
Remarks: Expected to be biodegradable.

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

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12.6. Other adverse effects
Biochemical Oxygen Demand (BOD) - 880mg/g

SECTION 13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods
This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber or to be disposed of by a licensed professional waste disposal company.

Contaminated packaging
Dispose of as the unused product.

SECTION 14. TRANSPORT INFORMATION

14.1 UN Number

DMSO	ADR/RID: -	IMDG: -	IATA: -
Acetic Acid	ADR/RID: 2789	IMDG: 2789	IATA: 2789

14.2 UN Proper Shipping Name

DMSO	ADR/RID:	Not Dangerous Goods
	IMDG:	Not Dangerous Goods
	IATA:	Not Dangerous Goods
Acetic Acid	ADR/RID:	ACETIC ACID, GLACIAL
	IMDG:	ACETIC ACID, GLACIAL
	IATA:	Acetic Acid, glacial

14.3 Transport hazard class (es)

DMSO	ADR/RID: -	IMDG: -	IATA: -
Acetic Acid	ADR/RID: 8 (3)	IMDG: 8 (3)	IATA: 8 (3)

14.4 Packing group

DMSO	ADR/RID: -	IMDG: -	IATA: -
Acetic Acid	ADR/RID: II	IMDG: II	IATA: II

14.5 Environmental hazards

	ADR/RID: No	IMDG Marine pollutant: No	IATA: No

14.6 Special precautions for user
No data available

SECTION 15. REGULATORY INFORMATION

This safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture
No data available

15.2 Chemical Safety Assessment
No data available

Please note that the label elements that used to go in Section 15 are now in Section 2.

SECTION 16. OTHER INFORMATION

The advice offered is derived from the current available information on the hazardous materials in this product and it component(s). Consideration has been made regarding the quantities offered in

the pre dispensed container. The advice offered is, therefore not all inclusive nor should it be taken as the descriptive of the compound generally.

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SAFETY DATA SHEET

Version: 1.0

Date written: 21st October 2013

SECTION 1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY / UNDERTAKING

Product Name	**2AA Dye**
Product Catalogue Name	**LT-2AA-03**
CAS-No.	**118-92-3**
Company:	Ludger Ltd Culham Science Centre Abingdon Oxfordshire OX14 3EB
Telephone:	01865 408554
Emergency Telephone:	01865 408554
Email:	info@ludger.com

SECTION 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture
Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]
Eye irritation (Category 2)

2.2 Label elements

Signal Word: Warning

Hazard Statement(s)

H319	Causes serious eye irritation.

Precautionary Statement(s)

P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do so. Continue rinsing.

2.3 Other hazard information:
None

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3. 1 Substances

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Synonyms: anthranilic acid
2-aminobenzoic acid
Formula: $C_7H_7NO_2$
Molecular weight: 137.14 g/mol

Component		Concentration
Name	2-AA Dye	-
CAS-No.	118-92-3	
EC-No.	204-287-5	

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures
General Advice
Consult a physician if exposure causes ill effects and if in any doubt. Show this safety data sheet to the physician/ first responder in attendance.

If ingested
Rinse mouth well with water. Never give anything by mouth if person has lost consciousness. Consult a physician.

In case of skin contact
Wash well with soap and water. Consult a physician.

If eyes are exposed
Rinse well with water/ eye wash solution for at least 15 minutes. Consult a physician. Show this safety data sheet to the physician/ first responder in attendance.

If inhaled
Move effected person(s) into fresh air. If not breathing, give artificial respiration. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

4.3 Indication of immediate medical attention and special treatment needed
No data available

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media
Use water spray, alcohol resistant foam, and dry chemical or carbon dioxide extinguishers.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, nitrogen oxides (NOx).

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

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Wear personal protective clothing when handling the chemical. Avoid dust formation. Avoid breathing in vapours, mist, dust or gas when clearing the chemical, work in a well ventilated area.

6.4 Environmental Precautions
Prevent any further leaking/ spillage if possible. Do not let the chemical enter the drainage system and discharge into the environment must be avoided.

6.5 Methods and material for containment and cleaning up
Gently sweep up the chemical, do not create dust, and put into a suitable container with a lid. Seal the container and arrange disposal.

6.4 Reference to other sections
See section 13 for information on disposal of the chemical.

SECTION 7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes and the formation of dust and aerosols. Provide appropriate exhaust ventilation when handling the chemical and if dust can be formed.

7.2 Conditions for safe storage, including any incompatibilities
Keep the container in a dry, cool and well ventilated place.

7.3 Specific end uses
No data available.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters
Contains no substances with occupational exposure limit values.

8.3 Exposure controls
Appropriate engineering controls
General advice is to always wear PPE when handling the chemical, in accordance with good laboratory practice. Wash hands after the removal of gloves.

Personal Protective Equipment
Eye / face protection
Safety glasses with side shields conforming to UN166. To have available equipment tested and approved under appropriate government standards such as NIOSH(US) or EN 166 (EU).

Skin protection
Handle with gloves. Following good laboratory practice the gloves should be checked for tears before use and proper glove removal technique to should be used when removing them. Dispose of used gloves as contaminated chemical waste. Wash and dry hands.
Gloves should be of the standard to satisfy the specifications of EU directive 89/686/EEC and the standard EN 374 derived form it.

Body Protection
Laboratory coat or a similar covering of the operators clothing.

Respiratory protection
If under extraction none is required.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

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Appearance	Form: Solid
Odour	No data available
Odour threshold	No data available
pH	No data available
Freezing/Melting Point	Melting point/range: 144-148°C ï lit.
Initial boiling point and boiling range	No data available
Flash Point	No data available
Evaporation rate	No data available
Flammability	No data available
Upper/lower flammability or explosive limits	No data available
Vapour Pressure	No data available
Relative Density	No data available
Solubility in water and solvents (mg/l)	No data available
Partition coefficient	No data available
Autoignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidising 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
No data available

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to Avoid
No data available

10.5 Incompatible materials
Strong oxidising agents.

10.6 Hazardous decomposition products
Other decomposition products ï No data available

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral ï rat ï 5,410 mg/kg
Remarks: Behavioural: Somnolence (general depressed activity), excitement and ataxia.
LC50 Inhalation ï rat ï 4h - >5.3mg/L
Skin corrosion/irritation
Skin ï rabbit ï No skin irritation.

Serious eye damage/irritation

Eyes ï rabbit ï Moderate eye irritation.

Respiratory or skin sensitisation
No data available.

Germ cell mutagenicity
Genotoxicity in vitro ï Not mutagenic in Ames test.
Histidine reversion (Ames)

Genotoxicity in vitro ï Human ï lymphocyte.
Mutation in mammalian somatic cells.

Genotoxicity in vivo ï mouse ï Intraperitioneal.
Sister chromatid exchange.

Carcinogenicity
Carcinogenicity ï rat ï Oral
Tumorigenic: Equivocal Tumorigenic agent by RTECS criteria. Kidney, Ureter, Bladder: Tumors
Carcinogenicity ï mouse ï Subcutaneous
Tumorigenic: Equivocal Tumorigenic agent by RTECS criteria. Lungs, Thorax or respiration: Bronchiogenic carcinoma. Liver: tumors.
This product is or contains a component that is not classifiable as to its carcinogenicity based on its IARC, ACGIH, NTP or EPA classification.
IARC: 3 ï Group 3: Not classifiable as to its carcinogenicity to humans (anthranilic acid)

Reproductive toxicity
Reproductive toxicity ï mouse ï Oral
Effects on fertility: Female fertility index (e.g. # females pregnant per #sperm positive females; # females pregnant per # females mated).

STOT-single exposure
No data available

STOT-repeated exposure
No data available

Aspiration hazard.
No data available

Potential Health Hazards

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through the skin. May cause skin irritation.
Eyes	Causes serious eye irritation.

Signs and symptoms of exposure
To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

Additional Information
RTECS: CB2450000

SECTION 12. ECOLOGICAL INFORMATION

12.1 Toxicity

Toxicity to fish	LC50 ï Pimephales promelas (Fathead minnow) ï 97 mg/l ï 96h

Toxicity to daphnia and other aquatic invertebrates.	EC50 ï Daphnia magna (Water flea) ï 85.7 mg/l ï 48h
Toxicity to algae	EC50 ï Desmodesmus subspicatus (Green algae) ï 31.3 mg/l ï 72h

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
Harmful to aquatic life.
No data available

SECTION 13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods
Waste can be burnt in a chemical incinerator equipped with an afterburner and scrubbers when first dissolved in a solvent, if impractical, seek a licensed disposal company for the disposal of waste materials.

Contaminated packaging
Treat packaging as unused product and dispose of with a licensed waste disposal company.

SECTION 14. TRANSPORT INFORMATION

14.1 UN Number
ADR/RID: - IMDG: - IATA: -

14.2 UN Proper Shipping Name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class (es)
ADR/RID: - IMDG: - IATA: -

14.4 Packing group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: No IMDG Marine pollutant: No IATA: No

14.6 Special precautions for user
No data available

SECTION 15. REGULATORY INFORMATION

This safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

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15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture
No data available

15.2 Chemical Safety Assessment
No data available

Please note that the label elements that used to go in Section 15 are now in Section 2.

SECTION 16. OTHER INFORMATION

The advice offered is derived from the current available information on the hazardous materials in this product and it component(s). Consideration has been made regarding the quantities offered in the pre dispensed container. The advice offered is, therefore not all inclusive nor should it be taken as the descriptive of the compound generally.

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Version: 1.0

Date written: 21st October 2013

SECTION 1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY / UNDERTAKING

Product Name	**2-Picoline Borane**
Product Catalogue Name	**LT-PB-01**
CAS-No:	**3999-38-0**
Company:	Ludger Ltd Culham Science Centre Abingdon Oxford OX14 3EB
Telephone:	01865 408554
Emergency Telephone:	01865 408554
Email:	info@ludger.com

SECTION 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]

Substances, which in contact with water, emit flammable gases (Category 2)
Skin irritation (Category 2)
Eye irritation (Category 2)
Specific target organ toxicity ï Single exposure (Category 3)

2.2 Label elements

Signal Word: Danger

Hazard Statement(s)

H261	In contact with water, releases flammable gas.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary Statement(s)

P231+P232	Handle under inert gas. Protect from moisture.
P261	Avoid breathing dust/ fume/gas/mist/vapours/spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do so. Continue rinsing.
P422	Store contents under inert gas.

2.3 Other hazard information:

No supplemental hazard statements.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

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3. 1 Substances

Synonyms: 2-picoline borane complex
2-Methylpyridine borane complex

Formula: $C_6H_{10}NB$
Molecular Weight: 106.96 g/mol

Component		Concentration
Name	2-picoline borane complex	100%
CAS-No.	3999-38-0	

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures

General Advice
Consult a physician if exposure causes ill effects and if in any doubt. Show this safety data sheet to the physician/ first responder in attendance.

If Ingested
Never give anything by mouth to an unconscious person. Rinse mouth well with water.

If skin is exposed
Wash area well with soap and water. Consult a physician.

If eyes are exposed
Rinse well with plenty of water for at 15 minutes and consult a physician.

If inhaled
Move the person into fresh air. If not breathing give artificial respiration. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2)

4.3 Indication of immediate medical attention and special treatment needed

No Data available

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media

Use a dry chemical extinguisher, as it is the only suitable extinguishing media.

5.2 Special hazards arising from the substance or mixture

Carbon oxides, nitrogen oxides (NOx), Borane/ boron oxides.

5.3 Advice for fire fighters

Fire fighters to wear self-contained breathing apparatus if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation in work areas. Evacuate personnel to safe areas to avoid breathing dust.

6.6 Environmental Precautions

Do not let the product enter the drains.

6.7 Methods and material for containment and cleaning up
Carefully sweep up the spill without creating any dust. Contain the collected material in a sealed suitable container, to await disposal. DO NOT USE WATER IN THE CLEANING PROCESS.

6.4 Reference to other sections
Please refer to section 13 for disposal of product and spills.

SECTION 7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Keep away from sources of ignition.

7.2 Conditions for safe storage, including any incompatibilities
Store in a cool, dark place. Keep the container tightly closed in a dry well ventilated place.

7.3 Specific end uses
No data available

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters
Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.4 Exposure controls
Appropriate engineering controls
Handle in accordance with good laboratory and safety practice. Wash hands before entering the laboratory and at the end of the workday/ when finished handling the material.

Personal Protective Equipment

Eye / face protection
Safety glasses. Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

Skin protection
Handle wearing gloves. Gloves must be inspected before use. Use proper glove removal technique (without the glove touching the skin) to avoid skin contact with the product. Dispose of contaminated gloves as chemical dry waste in accordance with applicable laws and good laboratory practices. Wash and dry the hands. Gloves must satisfy the specifications of EU directive 89/686/EEC and the standard EN 374 derived from it.

Body Protection
Laboratory coat or other types of body covering suitable for use in a laboratory.

Respiratory protection
When used under an operational fume hood no special protection is required. If required use respirators and components tested and approved under government standards such as NIOSH (US) or CEN (EU). Required level for nuisance exposure P98 (US) or P1 (EU EN 143), higher levels of protection OV/AG/P99 (US) or ABEK-P2 (EU EN 143).

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

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9.1 Information on basic physical and chemical properties

Appearance	Form: Solid Colour: White
Odour	No data available
Odour threshold	No data available
pH	No data available
Freezing/Melting Point	Melting point/ range: 44 - 46°C ï lit.
Initial boiling point and boiling range	No data available
Flash Point	100°C ï closed cup
Evaporation rate	No data available
Flammability	No data available
Upper/lower flammability or explosive limits	No data available
Vapour Pressure	No data available
Relative Density	No data available
Solubility in water and solvents (mg/l)	No data available
Partition coefficient: n- Octanol/water	No data available
Auto ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidising 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
Reacts violently with water.

10.4 Conditions to Avoid
Exposure to moisture.

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Other decomposition products - No data available

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects
Acute toxicity
No data available

Skin corrosion/irritation
No data available

Serious eye damage/irritation

No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

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

Reproductive toxicity
No data available

STOT-single exposure
Inhalation ï May cause respiratory irritation.

STOT-repeated exposure
No data available

Aspiration hazard.
No data available

Signs and symptoms of exposure
To the best of our knowledge the chemical, physical and toxicological properties have not been thoroughly investigated.

Additional Information
RTECS: Not 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
No data available

SECTION 13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods
Contact a licensed waste disposal service to collect/dispose of any waste material. Company should be advised to the nature of the substance, Highly Flammable.

Contaminated packaging

Treat as an unopened/ unused product.

SECTION 14. TRANSPORT INFORMATION

14.1 UN Number
ADR/RID: 2813 IMDG: 2813 IATA: 2813

14.2 UN Proper Shipping Name
ADR/RID: WATER-REACTIVE SOLID, N.O.S. (2-Picoline borane complex)
IMDG: WATER-REACTIVE SOLID, N.O.S. (2-Picoline borane complex)
IATA: Water-reactive solid, n.o.s. (2-Picoline borane complex)

14.3 Transport hazard class(es)
ADR/RID: 4.3 IMDG: 4.3 IATA: 4.3

14.4 Packing group
ADR/RID: II IMDG: II IATA: II

14.5 Environmental hazards
ADR/RID: No IMDG Marine pollutant: No IATA: No

14.6 Special precautions for user
No data available

SECTION 15. REGULATORY INFORMATION

This safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture
No data available

15.2 Chemical Safety Assessment
No data available

SECTION 16. OTHER INFORMATION

The advice offered is derived from the current available information on the hazardous materials in this product and it component(s). Consideration has been made regarding the quantities offered in the pre dispensed container. The advice offered is, therefore not all inclusive nor should it be taken as the descriptive of the compound generally.

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