Ludger Document # LC-EC50-96-Guide-v1.0

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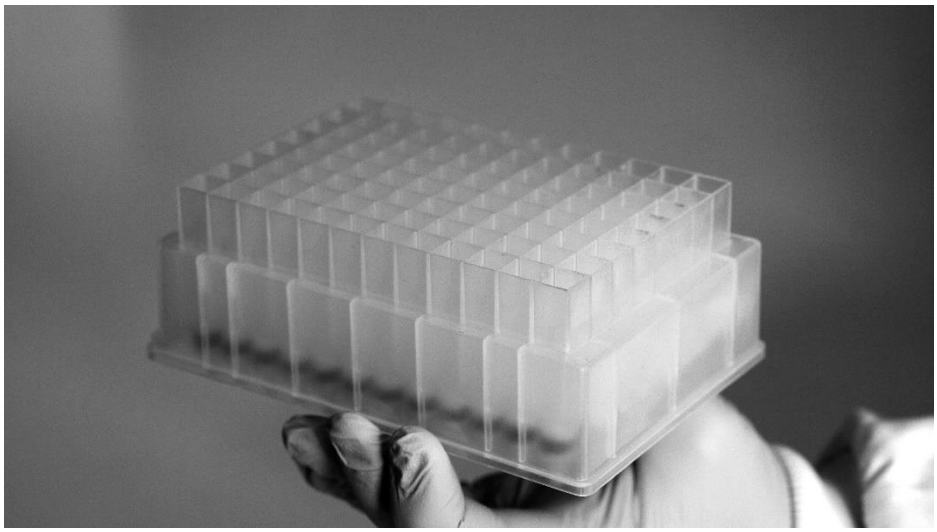
Contents

Specifications for the LudgerClean™ EC50 plate



Application: For purification of glycans from a variety of complex mixtures (including removal of salts and detergents).

Description: The plate contains a unique non-porous Electronic Interaction (EI) matrix. This acts like a super-hydrophobic resin that binds even very hydrophilic glycans. Most salts and detergents either simply pass through the plate or bind very lightly and can be washed off before the glycans are eluted.

Binding Capacity: Each well of the LudgerClean™ EC50 plate can typically bind up to 50 µg of O- or N-linked glycans.

Number of Samples: LudgerClean™ EC50 plates are designed for single use only.

Suitable Samples: A wide range of glycans can be purified. These include N-linked and O-linked type oligosaccharides, tri-saccharides and larger structures.
The plate is **not** suitable either for monosaccharides or disaccharides which are generally bound too weakly for efficient purification or for large linear poly-sialylated glycans which can be bound very tightly to the resin.
Glycan samples must be applied to the plate in solutions that are substantially aqueous.

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

Binding efficiency: > 95 % for most glycans

Binding Selectivity: Essentially stoichiometric binding and elution for most complex glycan mixtures.

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

Shipping: The product can be shipped at ambient temperature.

Handling: Ensure that any glass, plastic ware 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.

Safety: Please read the Safety Data Sheet (SDS) for all chemicals used.
All processes involving hazardous 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

For research use only. Not for human or drug use

Additional Reagents and Equipment Required

- Pure water (HPLC grade)
- Methanol
- 1M sodium hydroxide (aq)
- 30% acetic acid (aq)
- Acetonitrile (HPLC grade)
- Trifluoroacetic acid (Analar grade)
- Wash A: 5 % (v/v) acetonitrile (aq) plus 0.1% trifluoroacetic acid
- Wash B: 50 % (v/v) acetonitrile (aq) plus 0.1% trifluoroacetic acid
- Pipettes
- 0.5 µm or 0.2 µm microcentrifuge filters
- Microcentrifuge

Introduction

The LudgerClean™ EC50 plates have been designed for purification of glycans from non-carbohydrate material including salts, proteins, and detergents. Applications include clean-up of glycans following hydrazinolysis, enzyme treatment (including endoglycosidase and exoglycosidase digests) and before and after fluorescent labelling.

Outline of LudgerClean™ EC50 Clean-up Protocol

1 Wash the plate

The LudgerClean™ EC50 plate is washed with successive washes of methanol, 1 M sodium hydroxide, water, 30% acetic acid and water.

2 Prime the plate

The active surface of the resin in the plate is primed by washing with acidic aqueous solutions of acetonitrile.

3 Prepare the glycan sample

Dilute out any organic solvents and filter if required to remove viscous or particulate material from the sample.

4 Apply the glycan sample

The aqueous solution of glycan sample is applied to the plate.

5 Wash off the non-glycan contaminants

Non-glycan contaminants such as salts and detergents are washed out using dilute acidic aqueous acetonitrile.

6 Elute the glycans

Bound glycans are washed off the plate using a higher concentration of acidic aqueous acetonitrile.

7 Dry the eluted glycans (optional)

The eluted glycan solution can now be concentrated if required.

8 Analyse the glycans

The glycans are now ready for analysis.

Time Line for Clean-up

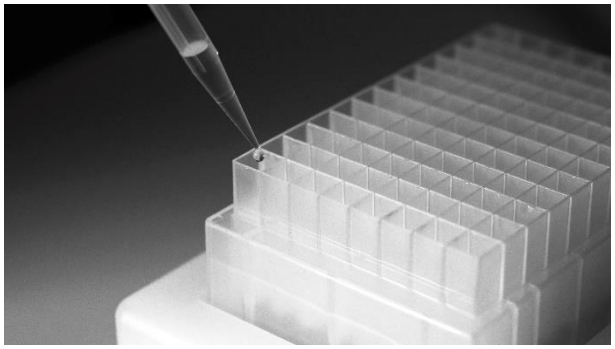
The LudgerClean™ EC50 glycan clean-up procedure typically takes around 120 minutes:

Procedure	Time	Elapsed Time (minutes)
Sample preparation	20 min	20
Wash and prime plate	50 min	70
Apply sample	20 min	90
Wash off non-glycan contaminants	15 min	105
Elute glycans	15 min	120

Instructions for Use with a Vacuum Manifold

Preparation of Plate

1 Wash the plate



Prepare each LudgerClean™ EC50 plate by washing with the following:

Reagent	Volume (mL)
methanol	0.5
1M sodium hydroxide	0.5
water	1
30% acetic acid	1
water	1

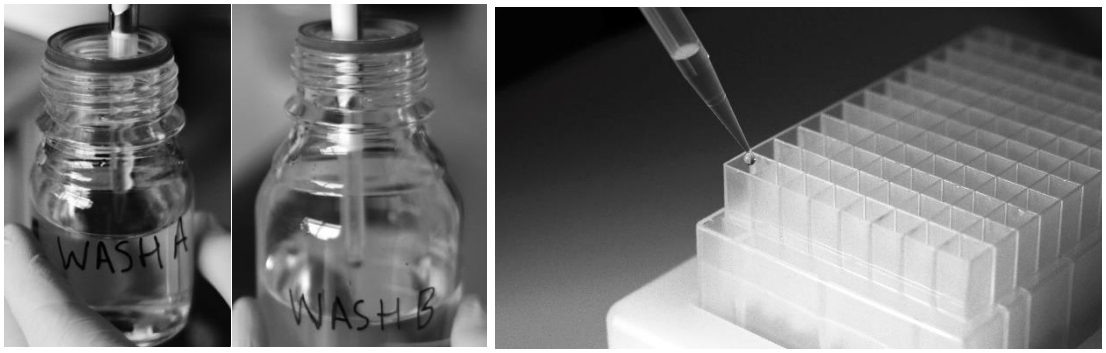
After each wash, apply a slow vacuum (approximately taking 5 minutes) to drain the plate using the vacuum manifold.

Allow each solvent to drain completely before adding the next one.

This removes any impurities that may have bound to the resin matrix during storage.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the plate (e.g. using a clean, gloved thumb) in order to resume normal flow. Alternatively, the plate can be covered with an LP-COLLPLATE-LID-96 to help the liquid to drain.

2 Prime the plate



Prime the plate with the following:

Reagent	Volume (mL)
Wash B	0.5
Wash A	1

After each wash, apply a slow vacuum (approximately taking 5 minutes) to drain the plate using the vacuum manifold.

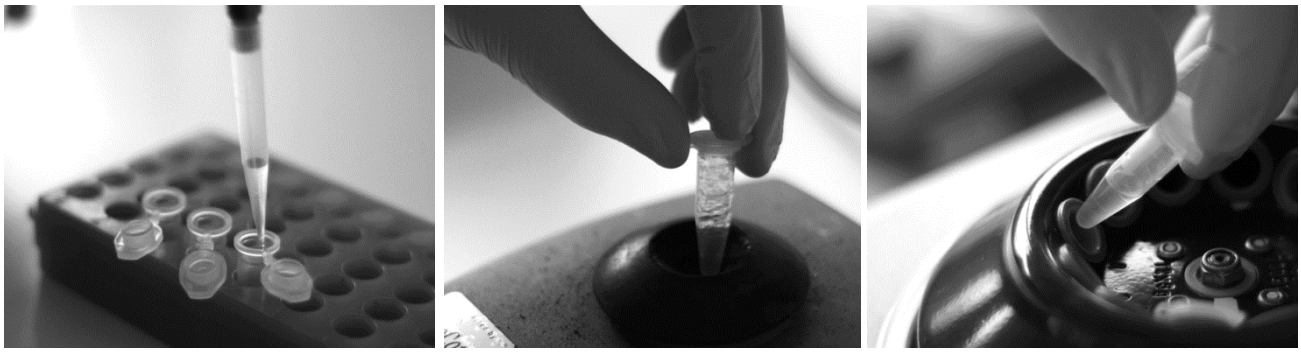
Allow each solvent to drain completely before adding the next one.

This prepares the surface of the resin for binding of the glycans.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the plate (e.g. using a clean, gloved thumb) in order to resume normal flow. Alternatively, the plate can be covered with an LP-COLLPLATE-LID-96 to help the liquid to drain.

Application of Sample and Removal of Contaminants

3 Prepare the glycan sample



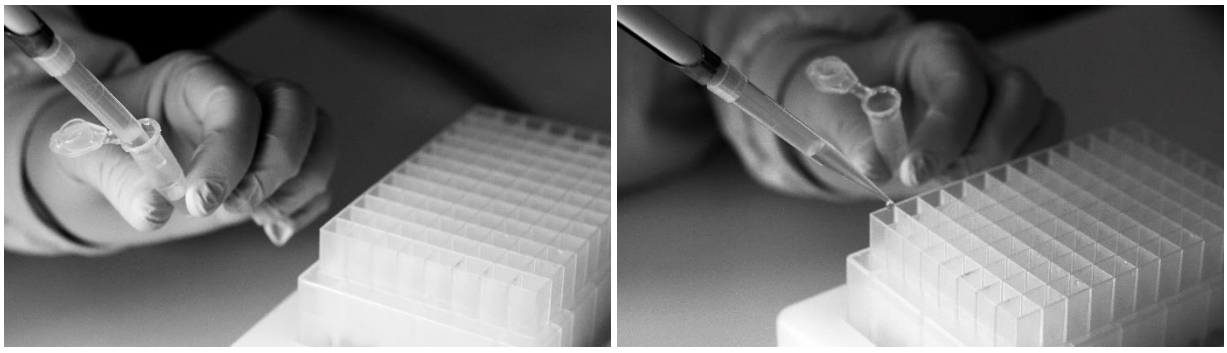
The sample to be cleaned must be in either an aqueous buffer or one containing only a low percentage of organic solvent. If the sample contains organic solvent then dilute with water until the organic solvent content is less than 5 % by volume.

Some types of sample may contain particulate or viscous material that can block the flow through the EC50 plate. These include some glycoproteins subjected to glycan release by endoglycosidase treatment. Monoclonal antibodies treated with PNGase F are particularly prone to formation of viscous material. In such cases, to minimize blockage of the EC50 clean-up plate:

a. Dilute each sample with 0.5 mL water.
b. Mix thoroughly by vortexing.
c. Spin in a micro-centrifuge (typical conditions are centrifugation at 10,000 rpm for 15 minutes).
d. Carefully pipette out the supernatant and apply to the prepared EC50 plate (see step 4).

We recommend that centrifugation is performed using 1.5 ml or 2 ml polypropylene microcentrifuge tubes.

4 Apply the glycan sample



Apply the sample to the plate.

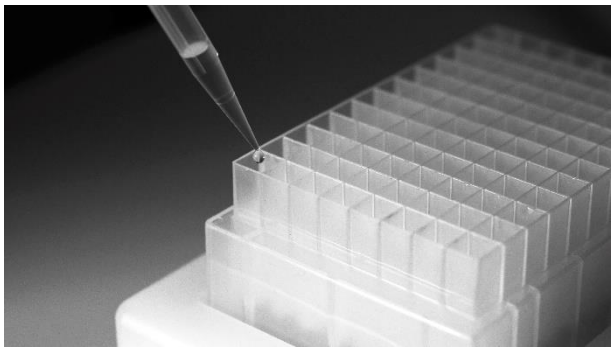
Load each sample onto a primed plate. Without applying a vacuum allow the sample to settle into the plate for 5 minutes. Next, apply a slow vacuum to drain the plate.

Allow sample to drain completely before going to the next step.

Glycans should bind to the matrix while salts and non-hydrophobic non-glycan contaminants pass through.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the plate (e.g. using a clean, gloved thumb) in order to resume normal flow. Alternatively, the plate can be covered with an LP-COLLPLATE-LID-96 to help the liquid to drain.

5 Wash off non-glycan contaminants

Wash the plate with the following:

Reagent	Volume (mL)
water	0.7
Wash A	0.7

After each wash, apply a slow vacuum (approximately taking 5 minutes) to drain the plate using the vacuum manifold.

Allow each solvent to drain completely before adding the next one.

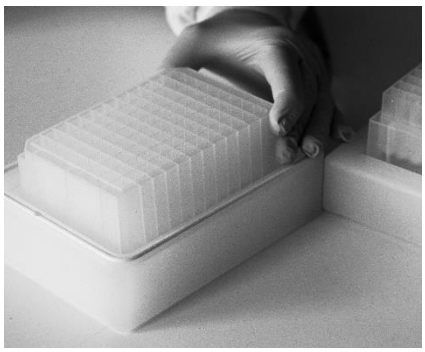
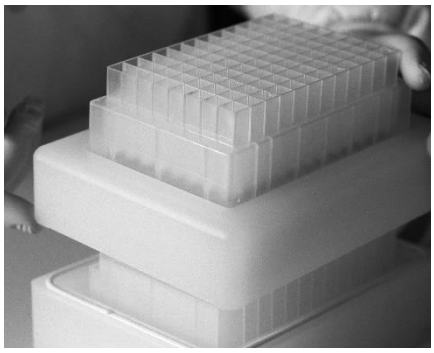
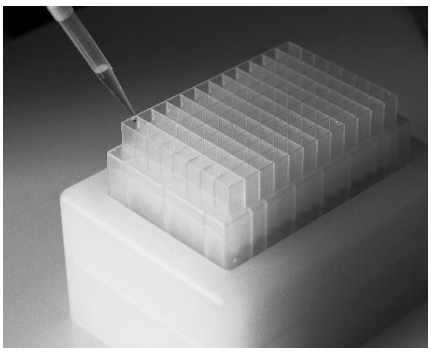
Discard these washes into a suitable waste container.

This removes residual salts and non-hydrophobic non-glycan material from the column.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the plate (e.g. using a clean, gloved thumb) in order to resume normal flow. Alternatively, the plate can be covered with an LP-COLLPLATE-LID-96 to help the liquid to drain.

Elution of Glycans

6 Elute the glycans

Place the plate over a collection vessel and recover the glycans by eluting with 4 x 0.2 mL of Wash B.

After each addition, apply a slow vacuum (approximately taking 5 minutes) to drain the plate using the vacuum manifold.

Allow each aliquot to drain before the next is applied.

Glycans should be eluted while hydrophobic material such as certain peptides, proteins, and detergents remain bound to the solid phase matrix.

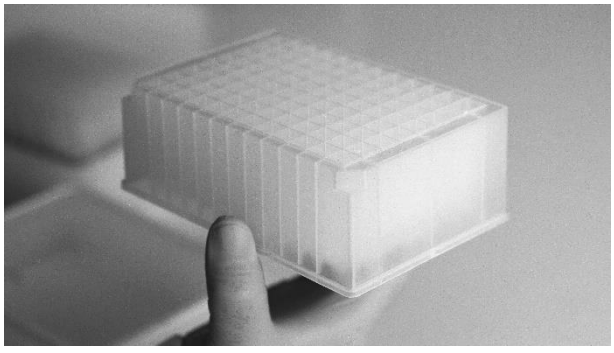
If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the plate (e.g. using a clean, gloved thumb) in order to resume normal flow. Alternatively, the plate can be covered with an LP-COLLPLATE-LID-96 to help the liquid to drain.

7 Dry the eluted glycans (optional)

If appropriate, evaporate the glycan containing fraction to dryness, then redissolve in a desired volume of water or solvent for further analysis.

8 Analyse the glycans

The glycans are now ready for analysis. Typical methods include 2AB or procainamide labelling.



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 # LC-EC50-96-Guide-v1.0

Ludger

SAFETY DATA SHEET

Version: 1.2

Date written/reviewed: 16th April 2019
Date reviewed: 3rd February 2021

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

Product Name	**LudgerClean EC50 cartridges/ LudgerClean EC50 plate**
Product Catalogue Name	**LC-EC50-24/LC-EC50-96**
CAS-No.	**7440-44-0**
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 (EU) No. 1272/2008 [EU-GHS/CLP]
This product has been classed as a non-hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements
The product does not require any labelling in accordance with EC directives or respective national laws.

Signal Word: None.

Hazard Statement(s)
None.

Precautionary Statement(s)
None.

2.3 Other hazard information:
None.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3. 1 Substances

Synonyms:	Carbon, Charcoal activated
Formula:	C
Molecular weight:	12.01 g/mol

Component		Concentration
Name	Carbon	-

CAS-No. 7440-44-0	
EC-No. 231-153-3	

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, if person is conscious. Do not give anything by mouth if unconscious.

If skin is exposed
Wash the affected area well with soap and water.

If eyes are exposed
Rinse eyes with water/ eye wash solution for at least 5 minutes as a precaution. If safe and easy to do so remove contact lenses and continue rinsing.

If inhaled
Move affected person to a source of ventilation/ fresh air. If not breathing give artificial respiration.

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 fully investigated.

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

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media
Select extinguishing media appropriate to surrounding area, compatible media's are water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
No data available.

5.3 Advice for firefighters
Wear self-contained breathing equipment, if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Avoid formation of dust and breathing it in. Wear PPE.

6.2 Environmental Precautions
None required.

6.3 Methods and material for containment and cleaning up
Use a damp cloth to sweep up the spilled product. Put the contaminated material and waste product into a suitable container with a lid and arrange disposal.

6.4 Reference to other sections
See Section 13 for more information on disposal.

SECTION 7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Handle the product wearing PPE, when used as part of clean up use under extraction, due to the nature of the chemicals used in the process, not the product itself.

7.2 Conditions for safe storage, including any incompatibilities
Store in a dry, well-ventilated area. Keep sealed in container until required preventing contamination.

7.3 Specific end uses
No data available.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters
Components with workplace control parameters
This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Handle the product wearing Personal protective equipment, wash and dry hands before and after handling the product. General good laboratory and safety practice.

Personal Protective Equipment
Eye / face protection
Wear eye protective equipment tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

Skin protection
Handle the product wearing gloves. Gloves are to satisfy the specifications of EU Directive 2016/425 and the standard EN 374 derived from it. Gloves to be checked for tares/holes before use and to be removed using the proper glove removal technique, so that the outer side of the gloves do not touch any skin. Gloves are to be disposed of as contaminated solid waste. See Section 13 for information on waste disposal.

Body Protection
Wear a laboratory coat or similar covering over the operators outside clothing.

Respiratory protection
Respiratory protection is not required with this product (on its own), when used for clean up the chemicals used require that the product is handled under extraction.

Thermal hazards
None.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Form: fine powder Colour: black
Odour	Odourless
Odour threshold	None
pH	6.0 – 9 at 40g/l at 25°C
Freezing/Melting Point	Melting point/range: 3,550°C – 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	<0.01 hPa at 20°C
Vapour Density	No data available
Relative Density	0.250 – 0.600 g/cm3
Solubility in water	Insoluble
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

Bulk Density	250 – 550 kg/m3 at 20°C

SECTION 10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available.

10.2 Chemical stability
The product is stable at the correct storage conditions.

10.3 Possibility of hazardous reactions
No data available.

10.4 Conditions to Avoid
High moisture and extreme temperatures.

10.5 Incompatible materials
Strong oxidizing agents.

10.6 Hazardous decomposition products
Other decomposition products.

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Intravenous – Mouse – 440 mg/kg

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 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
No data available.

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 irritation to the respiratory tract.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause irritation to the skin.
Eyes	May causes 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: FF5250100

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
Dispose of by using a licensed professional chemical liquid and solid waste disposal company. To be incinerated.

Contaminated packaging
Dispose of packaging as solid contaminated waste.

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: - IMDG: - IATA: -

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.