



**Product Guide for LudgerLiberate™  
Orela  
Glycan Release Kit**

**(Ludger Product Code: LL-ORELA-A2)**

**Ludger Document # LL-ORELA-A2-Guide-v2.2**

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## Specifications for LL-ORELA-A2

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<b>Application</b>	For release of O-linked glycans from glycoprotein therapeutics
<b>Description</b>	The kit contains reagents for the release of O-linked glycans from glycoprotein biopharmaceuticals. Released glycans have free reducing termini to allow fluorescent tagging by reductive amination.
<b>Number of Samples</b>	The kit contains reagents and materials for up to 12 glycoprotein samples analysed in parallel or two sets of 6 samples.
<b>Amount of Sample</b>	Typically, up to 1 mg of glycoprotein per sample.
<b>Suitable Samples</b>	Biopharmaceutical glycoproteins.
<b>Storage:</b>	Store refrigerated at 4 to 10 °C in the dark. If you have limited cold storage space, store the LudgerClean™ CEX-H cartridges (Cat # LC-CEX-H-01) at 4°C and the rest of the kit at room temperature. Protect from sources of heat, light, and moisture. The reagents are stable for at least 18 months from date of manufacture.
<b>Shipping:</b>	The product should be shipped at ambient temperature.

**For research use only. Not for human or drug use**

## Kit Contents



Each kit contains the following materials and reagents:

Cat. #	Item	Quantity
LL-ORELAREAGENT-01	Orela Release Reagent	2 x 3ml
LL-ACETIC-50P-01	Acetic Acid Solution	2 x 3 ml
LC-CEX-H-01	LudgerClean™ CEX-H cartridges	2 x 6 cartridges
LL-REACT-01	Glass reaction vials with PTFE lined caps	2 x 6 vials
LL-COLLV-01	Glass collection vials with PTFE lined caps	2 x 6 vials

## Additional Reagents and Equipment Required

- Pure water: resistivity 18 MΩ-cm, particle free (>0.22 μm), TOC <10 ppb
- Dialysis membranes, PD10 columns or similar for removal of salts and detergents from your glycoprotein samples\*
- Syringe (glass or PTFE) to transfer Orela reagent – e.g. 1 ml Hamilton or SGE glass syringe for liquids with teflon tipped plunger and stainless steel or PTFE needle. Do not use plastic syringes.
- Heating block, oven, or similar dry heater (a water bath cannot be used) that can be set between 40 and 100 °C
- Centrifugal evaporator (e.g. ThermoSavant SpeedVac® or GeneVac®). If using the ThermoSavant SpeedVac® we recommend the use of the Thermo Savant RH32-13 Rotor.
- Pipettes
- Optional - depending on your sample

## Safety and Handling

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- Please read the Material Safety Data Sheets (MSDS's) for all chemicals used (see Appendix 2).
- All processes involving the kit reagents should be performed using appropriate personal safety protection - eyeglasses, good quality chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard.
- 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.
- Once individual vials of reagents are opened, their contents should be used immediately and excess then discarded according to local safety rules.

## The Orela Reaction

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The Orela reaction involves the following steps:

1. **Liberation of the glycans as the glycosylamine derivative**  
The Orela reagent reacts at the link between the glycan and peptide backbone to liberate glycans as the glycosylamine.
2. **Conversion of the glycosylamine to the free glycans**  
The acid-labile glycosylamine derivative is hydrolysed to produce the free glycan.

## Time Line for Orela

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Procedure	Time
Start with pure glycoprotein samples	
Transfer samples to reaction vials and dry completely	24 hours
Add Orela release reagent	15 min
Incubate samples *	5 - 16 hour
Remove release reagent	2 hours
Acidification	overnight
Purification of released glycans	2 hours

\* The incubation time will vary depending on your particular glycoprotein samples. We recommend that at the start of a project you conduct a pilot study with an incubation time course to optimize the release conditions.

The following are typical incubation regimes:

O-Mode Orela (Fast):      Incubate 5 hours at 50°C  
O-Mode Orela (Normal):    Incubate 16 hours at 50°C

# Outline of the Orela Protocol

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- **Prepare the glycoconjugate**

Prepare the glycoprotein or glycopeptide samples by removing contaminants such as salts, detergents and dyes that could interfere with the labeling procedure.

- **Dry the glycans**

Place the samples in reaction vials and dry down.

- **Add release reagent to glycoconjugates**

Add Orela release reagent to each sample.

- **Incubate**

Incubate the samples to allow the release reaction to progress.

- **Acidification**

Add acetic acid solution and incubate at 4°C to convert glycosylamines into non-reduced glycans

- **Post-release cleanup**

Remove the peptide or protein using a cation exchange column

- **Store or analyse released glycans**

The released glycans are now ready for analysis

# Sample Preparation

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## 1 Purify the Glycoprotein

The glycopeptide or glycoprotein samples must be free of contaminants that can interfere with release reaction. These include the following:

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

Methods that are generally good for removal of such contaminants include the following:

- Dialysis against water or 0.1% trifluoroacetic acid (TFA) as some glycoproteins tend to precipitate in water
- Size exclusion chromatography using a small desalting column (e.g. PD10) with water or 0.1% TFA as eluant

## 2 Transfer Samples to Reaction Vials

The amount of sample for each reaction vial (cat # LL-REACT-01) should be in the range 50 µg to 1 mg.

*The reaction vials (5 ml glass vials with Teflon PTFE lined screw caps) included in the kit are pre-cleaned.*

## 3 Dry the Samples

Dry the samples using a centrifugal evaporator or a freeze drier.

*If freeze-drying, be careful to 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 can result in acid catalyzed loss of sialic acids (high temperatures, low pH) or uncontrolled glycan release (at high pH).*

# Release Reaction



## 4 Add Orela Reagent

Using a clean, dry glass or PTFE syringe with PTFE tipped plunger and teflon or stainless steel needle transfer 200  $\mu$ l of Orela release reagent (vial LL-ORELAREAGENT-01) to each dried sample.

Cap the reaction vials and mix by vortexing.

*This step must be performed in a chemical fume hood.*

*Ensure that the reaction vial caps are tightly screwed on. For extra security and to minimize you can seal the caps onto the vials using Parafilm, PTFE tape or similar.*

## 5 Orela Incubation

Place the reaction vials in a heating block, sand tray, or dry oven and incubate according to the type of glycan release you require:

O-Mode Orela (Fast):	Incubate 5 hours at 50°C
O-Mode Orela (Normal):	Incubate 16 hours at 50°C

*Use an oven or dry block - do not use a water bath.*

*The samples must be completely dissolved in the Orela reagent for efficient glycan release. To encourage complete dissolution the samples can be re-vortexed 15 and 30 minutes after the start of the incubation then the incubation continued.*

*During this step, the O-glycans are liberated from the glycoprotein as glycosylamine derivatives.*

*The kinetics of glycan release depends on the type of sample and the glycans. The incubation regimes above give good release for most samples. However, in some cases it may be useful to perform a time-course to optimize the release conditions. When performing a time-course, there are two factors to consider; (a) the yield and (b) side reactions (particularly peeling). The shorter the incubation time, the lower the peeling and lower the yield. Increasing the incubation time increases yield but can result in higher levels of peeling.*

## 6 Cool the Samples

After the incubation period, remove the samples from the incubation apparatus and allow them to cool completely to room temperature.

## Orela Reagent Removal

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### 7 Evaporate off Unreacted Orela Reagent

Remove unreacted Orela reagent by evaporation in a centrifugal evaporator.

*Use an evaporation chamber temperature of 30 to 40 °C.*

*N.B. Make sure that your centrifugal evaporator is rated to handle strong ammonia-like bases.. Your evaporator should be serviced and clean with good seals. Use an efficient cold trap with a temperature of 40°C or lower between the evaporation chamber and the pump. Dispose of the cold trap waste according to hazardous waste regulations. You can contact your local waste management service for advice.*

## Acidification

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### 8 Add 50% Acetic Acid (aq)

Add 200 µl of 50% acetic acid solution (vial LL-ACETIC-50P-01) to each sample, cap the reaction tube, vortex to mix.

### 9 Incubate for Acidification

Incubate in a refrigerator at 4°C overnight.

*This step allows acid catalyzed conversion of the glycosylamine derivatives to free unreduced glycans. The reaction is performed at 4°C to minimize acid catalyzed desialylation.*

# Glycan Purification

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## 10 Prime the LudgerClean™ CEX Cartridges

For each sample, prepare a LudgerClean™ CEX cartridge (cat # LC-CEX-H-01) by washing with 10 x 1 ml water

*If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.*

*Do not allow the resin to dry out.*

*Allow each aliquot to flow through the resin bed before the next solution is applied.*

## 11 Apply the Sample and Elute Glycans

- a. Place the cartridges over a collection vial (cat # LL-COLLV-01)
- b. Apply each sample to a prepared LudgerClean™ CEX cartridge (cat # LC-CEX-H-01) and allow the solution to flow through the resin bed slowly under gravity.
- c. Wash out each vial with 200 µl water and add to the top of each column.
- d. Further elute with 3 x 0.5 ml water.

*The eluted fluid will contain the purified, released glycans.*

*If the flow through the column is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.*

*Note that at the stage glycans will be in slightly acidic water after elution from the CEX cartridge.*

# Sample Storage

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## 12 Dry the Glycan Solutions

If required, the samples should be dried by centrifugal evaporation

*Keep the sample temperature <28 °C to minimize desialylation.*

*This step is optional and can be omitted if you are analyzing aliquots by any method that first involves drying (e.g. addition to a MALDI-MS plate or fluorescence labeling).*

### 13 Store the Glycans Frozen

For long-term storage, store the glycans at -20°C or lower temperature.

*The released glycans can be stored frozen either dried or after reconstitution with water.*

## Analysis of Released Glycans

The released glycans can be analyzed by a variety of techniques including the following:

- Fluorescence labeling with LudgerTag™ fluorophores followed by HPLC, CE or MS.  
The following table lists the current LudgerTag™ fluorophores and rates them according to the suitability for various analysis methods.

Fluorophore	HPLC	MS	CE
2-AB (2-aminobenzamide)	* * * * *	* * *	
2-AA (2-aminobenzoic acid)	* * * * *	* * * * *	* *
AA-Ac (3-(Acetylamino)-6-aminoacridine)	* * * * *	* * * * *	* * * * *
APTS (1-aminopyrene-3,6,8-trisulfonate)			* * * * *
2-AP (2-aminopyridine)	* * *	* *	

Key:

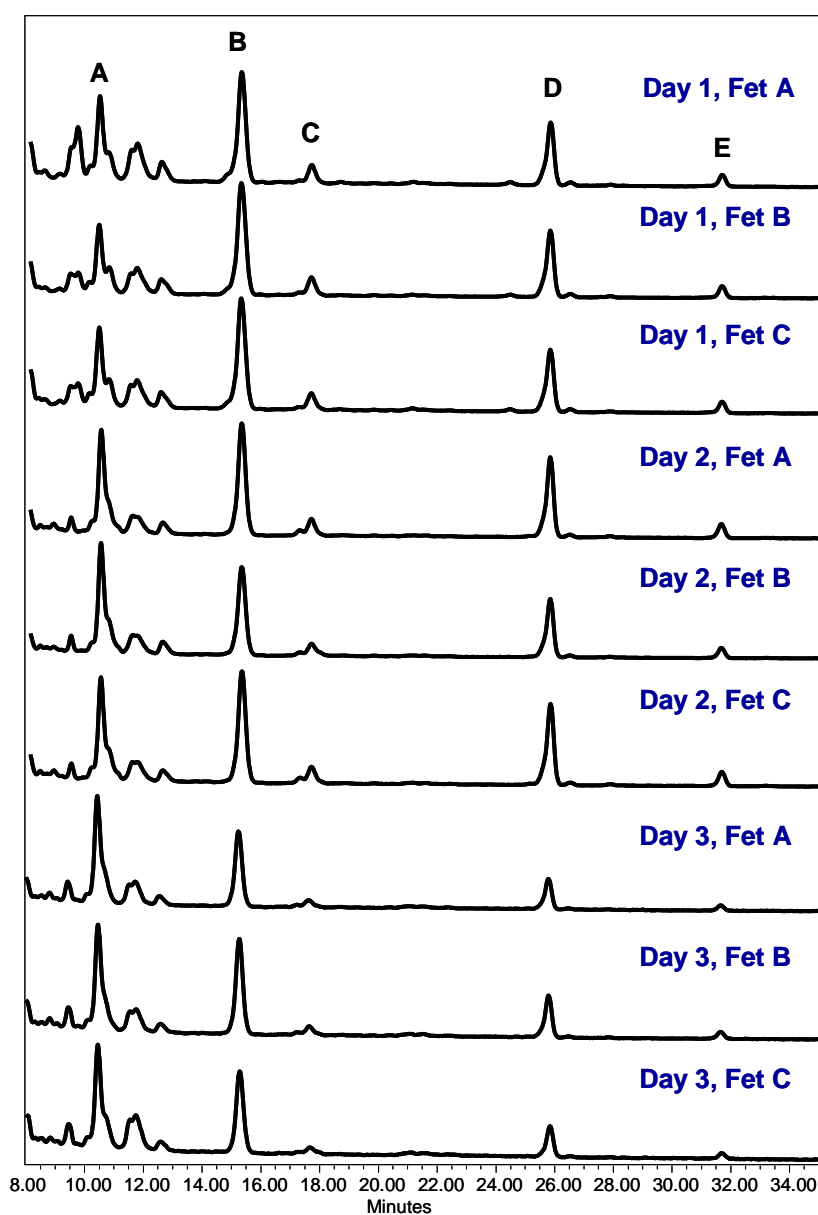
5 stars = excellent, 4 stars = good, 3 stars = fair, 1 - 2 stars = poor, no stars = not applicable

- Mass spectrometry
- HPLC-MS (high pH anion exchange chromatography with pulsed amperometric detection)

## Example Data: Repeatability using Bovine Fetuin

In order to obtain statistical data on the repeatability of the Orela glycan release kit, triplicate samples of bovine fetuin glycoprotein (GCP-FET-250) were subjected to Orela for release of the O-linked glycans. This method was repeated on three separate occasions. Released glycans were 2-aminobenzamide (2-AB) labeled and separated on a LudgerSep™N2 column (cat. No. LS-N2-4.6x150). The areas of each glycan peak were compared replicate to replicate and day to day.

Orela was performed using two separate conditions:- 50°C for 16 hours or 60°C for 6 hours.



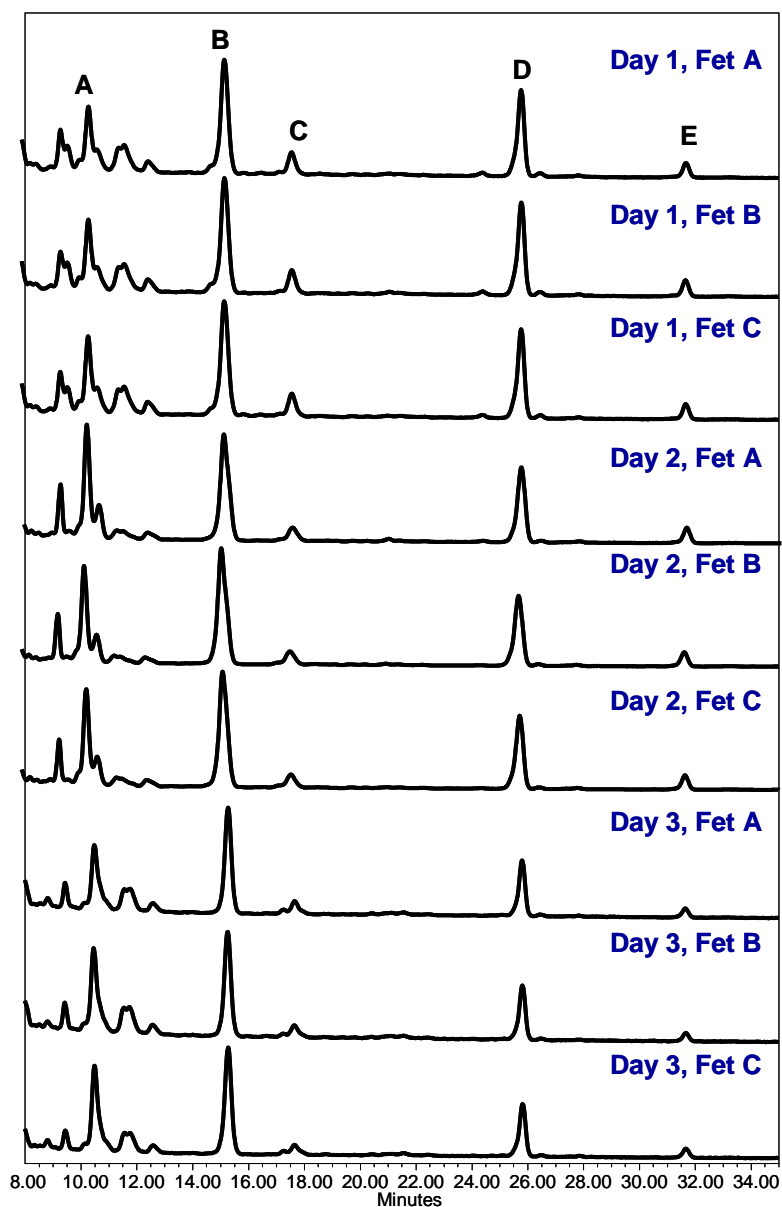
**Figure 1:** LudgerSep™N2 chromatograms showing 2-AB labeled glycans released from fetuin at 50°C for 16 hours.

Peak, GU	Peak A GU 2.29	Peak B GU 2.95	Peak C GU 3.26	Peak D GU 4.54	Peak E GU 5.59
% area					
Average	36.52	36.39	5.49	18.79	2.81
SD	8.43	3.85	1.08	4.30	0.76
CV	<b>23.09</b>	<b>10.57</b>	<b>19.68</b>	<b>22.87</b>	<b>26.87</b>

**Table 1:** % peak area data including the peeled peak: A, GU 2.29, for fetuin glycans released at 50°C for 16 hours.

Peak, GU	Peak B GU 2.95	Peak C GU 3.26	Peak D GU 4.54	Peak E GU 5.59
% area				
Average	57.74	8.59	29.30	4.38
SD	5.44	0.72	3.95	0.82
CV	<b>9.42</b>	<b>8.40</b>	<b>13.47</b>	<b>18.84</b>

**Table 2:** % peak area data not including the peeled peak: A, GU 2.29, for fetuin glycans released at 50°C for 16 hours.



**Figure 2:** LudgerSep™ N2 chromatograms showing 2-AB labeled glycans released from fetuin at 60°C for 6 hours.

Peak, GU	Peak A GU 2.29	Peak B GU 2.95	Peak C GU 3.26	Peak D GU 4.54	Peak E GU 5.59
% area					
Average	29.28	38.50	5.92	22.75	3.54
SD	3.89	1.69	1.03	3.02	0.52
CV	13.29	4.39	17.34	13.28	14.80

**Table 3:** % peak area data including the peeled peak: A, GU 2.29, for fetuin glycans released at 60°C for 6 hours.

Peak, GU	Peak B GU 2.95	Peak C GU 3.26	Peak D GU 4.54	Peak E GU 5.59
% area				
Average	54.58	8.34	32.08	5.00
SD	3.69	1.09	2.85	0.67
CV	6.77	13.04	8.88	13.46

**Table 4:** % peak area data not including the peeled peak: A, GU 2.29, for fetuin glycans released at 60°C for 6 hours.

## Warranties and Liabilities

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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 warranties, 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

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Document # LL-ORELA-A2-Guide-v2.2

## Appendix 1: Troubleshooting Guide

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The Orela 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.

### Low Yield

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#### **The temperature for Orela incubation 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 release period.

#### **The sample was incompletely solubilized.**

The glycoconjugate sample must be completely dissolved in the Orela reagent for maximum release efficiency. Please ensure that the sample is thoroughly mixed with the Orela reagent prior to the incubation and, as a precaution, re-mix the samples 15 and 30 minutes after the start of the incubation.

#### **The sample contained contaminants that interfered with the release**

Ensure that all samples are adequately purified before Orela release (see protocol step 1).

#### **There was less starting glycoprotein or glycopeptide than was originally estimated.**

#### **The glycans were lost during the sample workup**

Please ensure that the acidification and glycan purification steps are performed as in the protocol.

### Peeling of Glycans

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The peeling reaction is degradation of the released glycans characterized by loss of monosaccharide residues from the reducing terminus. O-glycans are generally more susceptible to peeling than N-glycans.

#### **The temperature-time profile for the Orela reaction was too harsh for the glycans**

Use the temperature-time profiles given in this protocol as a starting point. If you see peeling then for subsequent experiments reduce the temperature or time for the Orela reagent incubation.

## Desialylation of the Glycans

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

Avoid prolonged periods of exposure of sialylated glycan or glycoprotein samples in aqueous solutions at low pH and elevated temperatures.

In general, try to keep samples in solutions in the pH range 5 – 8.5 and avoid exposure to temperatures above 28 °C. Samples in pH buffered aqueous solutions (with pH between 5 and 8.5) tend to be resistant to acid catalyzed de-sialylation even at temperatures higher than 28°C. However, even then it is wise to err on the side of caution and keep the samples cool whenever possible.

## Cannot Assign Peaks on Samples Analyzed by HPLC, MS or CE

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### **Use glycoprotein and glycan standards appropriate for your project**

Select reference glycoprotein standards to use as positive controls for hydrazinolysis and use relevant glycan standards in subsequent analyses. Ludger is developing a range of matched glycoprotein and released glycans as certified reference standards for use in glycoprofiling studies. Please contact us for advice on what standards to use for your particular application.

## Appendix 2: Material Safety Data Sheets

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The advice offered in the following material safety data sheets (MSDS's) is derived from the currently available information on the hazardous materials in this product or component. 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 descriptive of the compound generally.

The following notes apply to all materials listed in the following MSDS's:

### **Transport information**

Contact Ludger for transportation information.

### **Abbreviations**

GLP                      Good Laboratory Practice



# SAFETY DATA SHEET

Version: 1.0

Date written: 24<sup>th</sup> February 2012

Date reviewed: 3<sup>rd</sup> March 2017

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

Product Name	<b>Orela Reagent</b>
Product Catalogue Name	<b>LL-ORELAREAGENT-01</b>
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]

Flammable liquids (Category 2)

Skin corrosion (Category 1A)

Specific target organ toxicity – single exposure (Category 3)

### Classification according to EU Directives 67/548/EEC or 1999/45/EC

Highly flammable. Causes burns. Irritating to respiratory system.

### 2.2 Label elements



Signal Word: Danger

### Hazard Statement(s)

H225

H314

H335

Highly flammable liquid and vapour.

Causes severe skin burns and eye damage.

May cause respiratory irritation.

### Precautionary Statement(s)

P210

smoking.

P261

P280

Keep away from heat/spark/open flames/hot surfaces – No

Avoid breathing dust/fume/gas/mist/vapours/spray.

Wear protective 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 easy to do so. Continue rinsing.

P310

Immediately call a POISON CENTRE or doctor/physician.

### 2.3 Other hazard information:

Lachrymator

## SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Synonyms: Orela Reagent

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## 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, if person is unconscious do not give them anything by mouth.

#### If skin is exposed

Remove any contaminated clothing. Wash the area well with plenty of soap and water. If chemical reaction is bad, consult a doctor.

#### If eyes are exposed

Rinse thoroughly with water/ eye wash solution, for at least 15 minutes. If present and possible, remove contact lenses and continue rinsing.

#### If inhaled

Move person into a source of fresh air/ventilation. If not breathing, give artificial respiration, consult a doctor.

### 4.2 Most important symptoms and effects, both acute and delayed

Shortness of breath, coughing, wheezing, laryngitis, head ache, nausea and vomiting. Spasm, inflammation and edema of the larynx and bronchi.

### 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

Select an extinguishing media appropriate to surrounding area. Compatible media for the product are water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

Carbon oxides, nitrogen oxides (NO<sub>x</sub>).

### 5.3 Advice for firefighters

Use water spray to cool unopened containers, wear self-contained breathing apparatus if necessary.

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## SECTION 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Ensure adequate ventilation; avoid breathing vapours, mist or gas. Remove any sources of ignition. Be aware that vapours can accumulate in low areas, potential for explosion. Move any unnecessary staff away from the spill.

### 6.2 Environmental Precautions

If safe and practical to do so, prevent any further spillage/leakage. Do not let the product enter the drainage system.

### 6.3 Methods and material for containment and cleaning up

Contain the spillage, a spill kit, mats or an inert material such as vermiculite would be advisable to use. Sweep up the contaminated material and store in a suitable container with a lid. Arrange disposal.

### 6.4 Reference to other sections

For more information on disposal of waste material, see Section 13.

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## SECTION 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Wash hands before and after handling the product. Avoid contact with skin and eyes, inhalation of mist/vapour. Keep away from sources of ignition.

### 7.2 Conditions for safe storage, including any incompatibilities

Store at 2 - 8°C, in a spark free refrigerator. Re-seal any opened containers and keep up right.

### 7.3 Specific end uses

No data available.

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## SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

Value	Control Parameter	Basis
STEL	6 ppm 11 mg/m <sup>3</sup>	UK. EH40 WEL - Workplace Exposure Limits
TWA	2 ppm 3.8 mg/m <sup>3</sup>	UK. EH40 WEL - Workplace Exposure Limits
TWA	5 ppm 9.4 mg/m <sup>3</sup>	Europe. Commission Directive 2000/39/EC establishing a first list of indicative occupational exposure limit values
remarks	Indicative	

### 8.2 Exposure controls

#### Appropriate engineering controls

When handling the product wear PPE, and wash hands, avoid contact with skin, eyes and clothing. Wash hands before and after handling the product.

#### Personal Protective Equipment

##### Eye / face protection

Wear safety glasses/goggles with side shields. Equipment used should be tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

##### Skin protection

Handle the product wearing gloves. Check gloves before use for holes etc. and to be removed after use, using the proper glove removal technique, to avoid any contact with the product and skin. Worn gloves to be treated as contaminated waste and disposed of according to good laboratory practices. Gloves must satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

**Body Protection**

Wear a laboratory coat or similar covering over clothing.

**Respiratory protection**

Handle the product under extraction, such a fume hood or cabinet.

**Thermal hazards**

No data available

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**SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES****9.1 Information on basic physical and chemical properties**

Appearance	Form: Liquid Colour: Pale yellow
Odour	No data available
Odour threshold	No data available
pH	>14.0 at 20°C
Freezing/Melting Point	Melting point/range:-74°C
Initial boiling point and boiling range	No data available
Flash Point	-26°C – closed cup
Evaporation rate	No data available
Flammability	No data available
Upper/lower flammability or explosive limits	Upper explosion limit: 12.8 % (V) Lower explosion limit: 3% (V)
Vapour Pressure	1,531 hPa at 50°C
Vapour Density	No data available
Relative Density	0.796 g/cm <sup>3</sup>
Solubility in water	No data available
Partition coefficient: n-Octanol/water	log Pow: -0.27
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

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**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. Extremes of temperature and direct sunlight.

Cleaning solutions containing strong acids, as acid reacts with the product, causing a reaction that produces a large amount of heat. The product causes corrosion in copper, aluminium, zinc and galvanised surfaces.

**10.5 Incompatible materials**

Cleaning solutions containing strong acids. Strong acids, Zinc, Tin/tin oxides.

#### **10.6 Hazardous decomposition products**

Other decomposition products – No data available.

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### **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 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** Harmful if inhaled. Material is destructive to the tissue of the mucous membranes and upper

respiratory tract. Causes respiratory tract irritation.

**Ingestion** Harmful if swallowed. Causes burns.

**Skin** Toxic if absorbed through skin. Causes skin burns.

**Eyes** Causes eye burns.

#### **Signs and symptoms of exposure**

Problems with respiratory system, burning sensation, shortness of breath, coughing, wheezing, headache, nausea and vomiting.

---

### **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 and arrange disposal of any waste product with a professional licensed disposal company. The waste product can be burnt in a chemical incinerator, fitted with an afterburner and scrubber, but it must be made clear that the material is highly flammable.

**Contaminated packaging**

Dispose of as contaminated solid waste.

---

**SECTION 14. TRANSPORT INFORMATION****14.1 UN Number**

ADR/RID: 2270

IMDG: 2270

IATA: 2270

**14.2 Transport hazard class (es)**

ADR/RID: 3 (8)

IMDG: 3 (8)

IATA: 3 (8)

**14.3 Packing group**

ADR/RID: II

IMDG: II

IATA: II

**14.4 Environmental hazards**

ADR/RID: No

IMDG Marine pollutant: No

IATA: No

**14.5 Special precautions for user**

No data available

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**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 its 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.



# SAFETY DATA SHEET

Version: 1.0

Date written: 28<sup>th</sup> February 2012Date reviewed: 3<sup>rd</sup> Mar 2017

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

Product Name	<b>Acetic Acid Solution, aqueous 50%</b>
Product Catalogue Name	<b>LL-ACETIC-50PC-01</b>
CAS-No.	<b>64-19-7</b>
Company:	Ludger Ltd Culham Science Centre Abingdon Oxfordshire OX14 3EB
Telephone:	01865 408554
Emergency Telephone:	01865 408554
Email:	info@ludger.com

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## SECTION 2. HAZARDS IDENTIFICATION

### 2.1 Classification of the substance or mixture

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

Skin irritation (Category 2)

Eye irritation (Category 2)

### 2.2 Label elements



Signal Word: Warning

### Hazard Statement(s)

H315

Causes skin irritation

H319

Causes serious eye irritation

### Precautionary Statement(s)

P305+P351+P338

contact

IF IN EYES: Rinse cautiously with water for several minutes. Remove lenses, if present and easy to so. Continue rinsing.

### 2.3 Other hazard information:

None.

---

## SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3. 1 Substances

Synonyms: acetic acid  
Formula:  $C_2H_4O_2$   
Molecular weight: 60.05 g/mol

Component		Classification	Concentration
Name	Acetic Acid	Flam. Liq. 3; Skin Corr. 1A;	50%
CAS-No.	64-19-7	H226, H314	
EC-No.	200-580-7		
Index-No.	6007-002-00-6		

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## 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 if the person is unconscious.

#### If skin is exposed

Remove any contaminated clothing/shoes. Wash effected area well with soap and water.

#### If eyes are exposed

Rinse thoroughly with water/eye wash solution for at least 15 minutes. Remove contact lenses, if present and is easy/safe to do so, continue with rinsing.

#### If inhaled

Move person into a source of fresh/ ventilation. If not breathing, give artificial respiration.

### 4.2 Most important symptoms and effects, both acute and delayed

Problems with breathing, including coughing, wheezing, shortness of breath. Headache, nausea, a burning sensation and the product is damaging to the respiratory system.

### 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

Select extinguishing media appropriate to surrounding area, compatible media of extinguishers are water spray, alcohol resistant foam, dry chemical and carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

Carbon oxides.

### 5.3 Advice for firefighters

If necessary wear self-contained breathing equipment.

---

## SECTION 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Wear PPE, Personal protective equipment. Avoid breathing in vapours, gas or mist. Ensure adequate ventilation and move any non-essential staff away from spillage.

### 6.4 Environmental Precautions

Do not let the product enter the drainage system.

### 6.5 Methods and material for containment and cleaning up

Contain the spillage by using spillage mats or inert material such as vermiculite. Collect the contaminated material and put into a suitable container, with a lid and arrange for collection and disposal.

### 6.4 Reference to other sections

For more information on disposal, see section 13.

---

## SECTION 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin, eyes and breathing in vapour, mist or gas.

### 7.2 Conditions for safe storage, including any incompatibilities

Store in a cool, well ventilated cabinet.

### 7.3 Specific end uses

No data available.

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## SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

Components with workplace control parameters.

Component	CAS-No.	Value	Control Parameters	Basis
Acetic Acid	64-19-7	TWA	10ppm 25mg/m <sup>3</sup>	Europe. Commission Directive 91/322/EEC on establishing indicative limit values.
	Remarks	Indicative		

### 8.3 Exposure controls

#### Appropriate engineering controls

Wash and dry hands before and after handling the product, in accordance to good laboratory and safety practice.

#### Personal Protective Equipment

##### Eye / face protection

Wear safety glasses/ goggles, when handling the product. These should be tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

##### Skin protection

Handle the product wearing gloves. Gloves to be checked before use, for rips and tares, when removed the proper technique in removing them should be used, so no skin comes into contact with the outside of the glove. Gloves should be disposed as solid contaminated waste. Gloves must satisfy the specifications of the EU Directive 89/686/EEC and the standard EN 374 derived from it.

##### Body Protection

Wear a laboratory coat or similar covering over clothes, when handling the product.

##### Respiratory protection

Handle the product under extraction from a fume cabinet or extraction hood.

##### Thermal hazards

No data available

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## SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

Appearance	Form: Liquid
Odour	No data available
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	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

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## 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

Oxidizing materials, Metals, Amines, Alcohols, Peroxides, permanganates, e.g. potassium permanganate, soluble carbonates and phosphates, Hydroxides.

### 10.6 Hazardous decomposition products

Other decomposition products - No data available

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## 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**

No data available

**STOT-repeated exposure**

No data available

**Aspiration hazard.**

No data available

**Potential Health Hazards****Inhalation**

tissue of the

May be harmful if inhaled. Material is extremely destructive to the

mucous membranes and upper respiratory tract.

**Ingestion**

May be harmful if swallowed. Causes burns.

**Skin**

May be harmful if absorbed through skin. Causes skin burns.

**Eyes**

Causes eye burns.

**Signs and symptoms of exposure**

Material can cause problems with the respiratory tract, such as burning sensations, coughing, wheezing and laryngitis, breathing problems, Headache and nausea. Eyes and skin, burns.

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**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

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**SECTION 13. DISPOSAL CONSIDERATIONS**

**13.1 Waste treatment methods**

Contact a licensed professional waste disposal company, for solid and liquid chemical waste. To collect and dispose of any waste product.

**Contaminated packaging**

Dispose of as contaminated solid waste or as unused product.

---

**SECTION 14. TRANSPORT INFORMATION****14.1 UN Number**

ADR/RID: 2790

IMDG: 2790

IATA: 2790

**14.2 UN Proper Shipping Name**

ADR/RID: ACETIC ACID SOLUTION

IMDG: ACETIC ACID, SOLUTION

IATA: Acetic Acid Solution

**14.3 Transport hazard class(es)**

ADR/RID: 8

IMDG: 8

IATA: 8

**14.4 Packing group**

ADR/RID: III

IMDG: III

IATA: III

**14.5 Environmental hazards**

ADR/RID: No

IMDG Marine pollutant: No

IATA: No

**14.6 Special precautions for user**

No data available

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**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 its 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.



# SAFETY DATA SHEET

Version: 1.1

Date written: 22<sup>nd</sup> March 2012

Date reviewed: 3<sup>rd</sup> March 2017

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

Product Name **Ludgerclean cartridges with 1M HCl**

Product Catalogue Name **LC-CEX-A6, LC-CEX-H-01**

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]**

Skin corrosion (category 1B)

Specific target organ toxicity – single exposure (Category 3)

### 2.2 Label elements



Signal Word: Danger

### Hazard Statement(s)

H314 Causes severe skin burns and eye damage.

H335 May cause respiratory irritation.

### Precautionary Statement(s)

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P305+P315+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. 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: Hydrochloric acid: HCl  
Resin: AG Cation exchange resin, hydrogen form

Formula: Hydrochloric acid: HCl  
Molecular Weight: HCl: 36.46g/mol

Component		Classification	Concentration
Name	CEX Resin	-	> 97 %
CAS-No.	none		
EC-No.	none		
2 <sup>nd</sup> Name	Hydrochloric Acid	Skin Corr. 1B; STOT SE3; H314,	1 – 3 %
CAS-No.	7647-01-0	H335	
EC-No.	231-595-7		
Index-No.	017-002-01-X		

For the full text of the H-statements mentioned in this section, Section 2 and 16.

## 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. Do not give anything by mouth if person is unconscious. Rinse mouth well with water.

#### If skin is exposed

Remove contaminated clothing and shoes immediately. Wash area well with plenty of soap and water.

#### If eyes are exposed

Rinse thoroughly with water or eye wash, for at least 15 minutes. Remove contact lenses if present and continue rinsing.

#### If inhaled

Remove person to a source of fresh air/ ventilation. If not breathing, give artificial respiration.

### 4.2 Most important symptoms and effects, both acute and delayed

Burning sensation, coughing and difficulties breathing. This product can be destructive to tissue of the mucous membranes and upper respiratory tract, eyes and skin.

### 4.3 Indication of immediate medical attention and special treatment needed

No data available.

## SECTION 5. FIRE-FIGHTING MEASURES

### 5.1 Extinguishing media

Select an extinguisher that media is compatible with the surrounding of the fire. Compatible fire extinguisher media are Carbon dioxide, alcohol resistant foam, water spray.

### 5.2 Special hazards arising from the substance or mixture

Hydrogen Chloride gas

### 5.3 Advice for firefighters

If necessary, fire fighters are to wear self-contained breathing apparatus.

## SECTION 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Wear personal protective equipment. Avoid breathing in vapours, mist or gas by ensuring adequate ventilation. Move any unrequired staff away from the spill area.

### 6.6 Environmental Precautions

Prevent any further leakage if practical and safe to do so. Do not let the product enter the drainage system.

### 6.7 Methods and material for containment and cleaning up

Soak up the spillage by using an inert absorbent material, such as vermiculite. Collect the waste material and store in a suitable container with a lid, arrange for collection and disposal.

### 6.4 Reference to other sections

For information on disposal see Section 13.

## SECTION 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin, eyes and inhalation of vapour or mist. Wear PPE.

### 7.2 Conditions for safe storage, including any incompatibilities

Store in cool, dry, well ventilated place at 2 – 8 °C. Cartridges must be stored horizontally.

### 7.3 Specific end uses

No data available.

## SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Hydrochloric Acid	7647-01-0	TWA	5 ppm 8 mg/m <sup>3</sup>	Europe. Commission Directive 2009/39/EC establishing a first list of inductive occupational limit values.
	Remarks	Indicative		
		STEL	10 ppm 15 mg/m <sup>3</sup>	Europe. Commission Directive 2009/39/EC establishing a first list of inductive occupational limit values.
		Indicative		
		TWA	1 ppm 2 mg/m <sup>3</sup>	UK. EH40 WEL- Workplace Exposure Limits
		Indicative		
		STEL	5 ppm 8 mg/m <sup>3</sup>	UK. EH40 WEL- Workplace Exposure Limits

## 8.4 Exposure controls

### Appropriate engineering controls

Handle the product following good laboratory and safety practice. Wash hands before and after handling the product, even with wearing gloves.

### Personal Protective Equipment

#### Eye / face protection

Wear fitted safety goggles/ glasses when handling the product. Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

#### Skin protection

Wear gloves when handling the product. Gloves must be inspected before use for tears/ holes and proper glove removal technique to be employed, to avoid skin contact with the product. Dispose of used gloves as contaminated waste (See section 13), wash and dry hands. Gloves must satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

#### Body Protection

Wear a laboratory coat or similar covering over clothing when handling the product.

#### Respiratory protection

Handle the product whilst using a fume cupboard / extraction hood.

#### Thermal hazards

No data available.

---

## SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

Appearance	Form: Solid and Liquid
	Colour: Pale orange
Odour	Pungent
Odour threshold	No data available
pH	No data available
Freezing/Melting Point	-30°C
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
Vapour Density	No data available
Relative Density	No data available
Solubility in water	Fully miscible.
Partition coefficient	No data available
Autoignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	None
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 when stored in recommended conditions.

**10.3 Possibility of hazardous reactions**

No data available

**10.4 Conditions to Avoid**

Excessive humidity and heat. Store at correct temperature, 2 – 8 °C.

**10.5 Incompatible materials**

Bases, Amines, Alkali metals, Metals, permanganates, e.g. potassium permanganate, Fluorine, metal acetylides, hexalithium disilicide.

**10.6 Hazardous decomposition products**

Other decomposition products - No data available

---

**SECTION 11. TOXICOLOGICAL INFORMATION****11.1 Information on toxicological effects****Acute toxicity**

Hydrochloric acid: LD50 Oral – Rabbit – 900mg/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**

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 – Group 3: Not classifiable as to its carcinogenicity to humans.

**Reproductive toxicity**

No data available

**STOT-single exposure**

Inhalation – May cause respiratory irritation.

**STOT-repeated exposure**

No data available

**Aspiration hazard.**

No data available

**Potential Health Hazards**

**Inhalation** Harmful if inhaled. Material is destructive to the tissue of the mucous membranes and upper respiratory tract.

<b>Ingestion</b>	Harmful if swallowed. Causes burns.
<b>Skin</b>	Harmful if absorbed through the skin. Causes skin burns.
<b>Eyes</b>	Causes burns to the eyes.

**Signs and symptoms of exposure**

Burning sensation, coughing, breathing problems, inflammation of larynx and bronchi. The product is destructive to the tissue of the mucous membranes and upper respiratory tract, eyes and skin.

**Additional Information**

RTECS: MW4025000

---

**SECTION 12. ECOLOGICAL INFORMATION****12.1 Toxicity**

Hydrochloric acid: Toxicity to Fish

LC50 – Gambusia affinis (mosquito Fish) – 282 mg/l – 96h

**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 professional disposal company of waste chemical (solid and liquid) materials, to arrange collection and disposal of waste product.

**Contaminated packaging**

Dispose of as unused product.

---

**SECTION 14. TRANSPORT INFORMATION**

This information is for HCl as the CEX resin has no classification.

**14.1 UN Number**

ADR/RID: 1789

IMDG: 1789

IATA: 1789

**14.2 UN Proper Shipping Name**

ADR/RID: HYDROCHLORIC ACID

IMDG: HYDROCHLORIC ACID

IATA: Hydrochloric Acid

**14.3 Transport hazard class(es)**

ADR/RID: 8

IMDG: 8

IATA: 8

**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

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 its 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.

**Hazard Statement(s)**

H314

Causes severe skin burns and eye damage.

H335

May cause respiratory irritation.

**Precautionary Statement(s)**

P261

Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P280

Wear protective gloves/ protective clothing/ eye protection/ face

protection.

P305+P315+P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove

contact

lenses, if present and easy to do. Continue rinsing.

P310

Immediately call a POISON CENTRE or doctor/ physician.