



Product # LZ-ACASE-KIT

Ludger Document # LZ-ACASE-KIT-Guide-v1.1

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Specifications for LZ-ACASE-KIT

Description Acetyl esterase (sialate-O-acetylesterase) is a recombinant protein from *Tannerella*

forsythia, ATCC 43037 strain, expressed in *Escherichia coli*. The enzyme removes acetyl groups attached via an O- group, mainly 9-, 8- and 7-. It can be used for monitoring of

diacetylation of sialic acids on products such as erythropoietin (EPO).

Application Acetyl esterase (sialate-O-acetylesterase) can be used to remove 9-, 8- and 7-O-acetyl

groups from released sialic acids, released glycans or glycoproteins.

Enzyme Commission Number EC 3.1.1.53

Number of Samples Sufficient for up to 50 samples.

Amount of Sample Up to 10 µg glycoprotein, up to 2.5 µg released glycans and up to 1 µg free sialic acid

per digestion.

Suitable Samples Acetyl esterase (sialate-O-acetylesterase) can act upon complex glycoprotein samples,

such as erythropoietin (EPO), bovine submaxillary mucin and oral epithelial cell-bound glycans, and on N- and O-glycans released from a glycoprotein. Either fluorescently labelled or unlabelled glycans are suitable. It can also be used on released sialic acids.

Unit Definition One unit (U) of acetyl esterase is defined as the amount of enzyme required to produce

300 µmole of 4-nitrophenol and acetate in 1 minute at 30°C in a buffer containing 50 mM Tris-HCl, 140 mM NaCl, pH 8.5, from 4-nitrophenyl acetate, a chromogenic

esterase substrate.

Molecular Weight 76.3 kDa.

Storage Store at 4°C. Protect from sources of heat and light. When stored correctly, the

enzyme should be stable for 24 months from date of purchase. Exposure to ambient temperatures $(20 - 26^{\circ}\text{C})$ over 3 days does not result in a reduction of enzymatic

activity.

Shipping The product should be shipped at 4°C.

Handling Ensure that any glass, plastic ware or solvents used with this item are free of

environmental carbohydrates. Use powder-free gloves for all sample handling

procedures and avoid contamination with environmental carbohydrate.



Safety

For research use only. Not for human or drug use

Please read the Safety Data Sheets (SDSs) for all chemicals used. All processes involving labelling reagents should be performed using appropriate personal safety protection – safety glasses, chemically resistant gloves (e.g. nitrile), lab coat, and when appropriate, in a laboratory fume cupboard.



Kit Contents

The kit contains the following materials and reagents:

Cat. #	Item	Quantity
LZ-ACASE-50	LudgerZyme Acetyl Esterase (Supplied in PBS pH7.5 buffer containing 10 mM Tris-Ho	1 CI)
LZ-ACASE-BUFF	LudgerZyme Acetyl Esterase RXN 10x buffer (500 mM sodium acetate pH5.5)	1

Additional Reagents and Equipment Required

- Pure water: resistivity above 18 M Ω -cm, particle free (>0.22 μ m), TOC <10 ppb.
- Microcentrifuge tubes for reaction.
- Waterbath or oven with constant temperature maintenance at 37°C.
- Pipettes and tips.

Time Line for Procedure

ProcedureApprox. TimePreparing assay mixture5 minIncubationup to 16 hrs



Method

1 Preparing assay mixture

To digest <10 μ g of glycoprotein, make up a total reaction volume of 10 μ L by dissolving the glycoprotein in 1 μ L of LudgerZyme Acetyl Esterase RXN 10× buffer, 1 μ L of LudgerZyme Acetyl Esterase and water to a final volume of 10 μ L.

Use the same method for digestion of <2.5 µg released glycans or <1 µg free sialic acid.

The reaction may be scaled-up linearly to accommodate larger amounts of glycoprotein, released glycans or free sialic acids.

2 Incubation

Incubate your samples in a water bath, oven or any other constant heating source at 37°C for 7 to 16 hours.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

The samples are now ready for clean-up and subsequent analysis by HPLC.

Optionally, the samples can be stored frozen at this point for analysis at a later date

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 # LZ-ACASE-KIT, version v1.1

Appendix 1: Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the acetyl esterase kit for the removal of 9-O-acetyl groups from sialic acids.

The positive control gives negative results

The enzyme became inactive

Exposure to temperature higher than ambient temperature can render the enzyme inactive. To avoid loss of activity, store enzyme under the recommended conditions.

The 9-O-acetyl groups are released inefficiently

The glycoproteins are not dissolved

If solubilization of glycoproteins is insufficient, digestion will be incomplete.

To ensure sample is dissolved properly, vortex sample longer or make up digestion in a larger volume of reaction mixture.

The sample contained contaminants that interfered with acetyl esterase activity

Please ensure that the glycoproteins are free from contaminants (e.g. SDS) before digestion. Some buffers can cause rearrangement of the O-acetyl group on the sialic acids.

The incubation condition 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 period.

There was less starting glycoproteins than was originally estimated

Please ensure sufficient amount of sample is used.



SAFETY DATA SHEET

Version: 1.0 Date written: 2 Dec 2015

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

Product Name Acetyl esterase (in 8mM Na₂HPO₄, 8mM NaH₂PO₄, 148mM NaCl and

10mM Tris-base, pH 7.4)

Product Catalogue Name LZ-ACASE-50

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

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008. This substance is not classified as dangerous according to Directive 67/548/EEC.

2.2 Label elements

The product does not need to be labelled in accordance with EC directives or respective national laws.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3. 1 Substances

Synonyms: Disodium hydrogen phosphate

sec-Sodium phosphate Disodium phosphate

Sodium hydrogenphosphate

Formula: Na₂HPO₄

Molecular Weight: 141.96 g/mol

Synonyms: Monosodium phosphate

Sodium dihydrogen phosphate

Formula: NaH₂PO₄

Molecular weight: 119.98 g/mol

EC-No.: 231-449-2

Formula: NaCl

Molecular Weight: 58.44 g/mol

CAS-No.: 7647-14-5 EC-No.: 231-598-3



Formula: C₄H₁₁NO₃

Molecular Weight: 121.14 g/mol

CAS-No.: 77-86-1 EC-No.: 201-064-4

No components need to be disclosed according to the applicable regulations.

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Oxides of phosphorus, Hydrogen chloride gas, Potassium oxides, Sodium oxides, Carbon oxides, nitrogen oxides (NOx)

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust. For personal protection see section 8.



6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

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.

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Storage class (TRGS 510): Non Combustible Solids

Hygroscopic

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls

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

Personal protective equipment

Eye/face protection

Safety glasses with side-shields conforming to EN166 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. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Body Protection

Impervious clothing. Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.



Respiratory protection

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

Do not let product enter drains.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

solid, crystalline Appearance Form Odour No data available Odour Threshold No data available Hq 7.2 - 7.6 at 25 °C Melting point/freezingpoint No data available Initial boiling point andboiling range No data available Flash point Not applicable **Evaporation rate** No data available Flammability (solid, gas) No data available Upper/lower flammability or explosive limits No data available Vapour pressure No data available Vapour density No data available Relative density No data available Water solubility 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 Oxidizing properties No data available

9.2 Other safety information

No data available

SECTION 10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

No data available

10.5 Incompatible materials

Strong oxidizing agents, Strong acids

10.6 Hazardous decomposition products

Other decomposition products - No data available In the event of fire: see section 5



SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - rat - > 3,000 mg/kg LD50 Dermal - rat - > 5,000 mg/kg (OECD Test Guideline 402)

Skin corrosion/irritation

Skin - rabbit

Result: No skin irritation (OECD Test Guideline 404)

Serious eye damage/eye irritation

Eyes - rabbit

Result: No eye irritation (OECD Test Guideline 405)

Respiratory or skin sensitisation

Buehler Test - guinea pig

Does not cause skin sensitisation.

(OECD Test Guideline 406)

Germ cell mutagenicity

Result: Not mutagenic in Ames Test.

in vitro assay Result: negative

In vitro tests did not show mutagenic effectsResult: In vivo tests did not show any chromosomal

changes.

Carcinogenicity

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

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

Additional Information

RTECS: Not available

Vomiting, Diarrhoea, Dehydration and congestion may occur in internal organs. Hypertonic salt solutions can produce inflammatory reactions in the gastrointestinal tract., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Repeated dose toxicity - rat - Oral - No observed adverse effect level - 1,000 mg/kg

RTECS: TY2900000

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

SECTION 12. ECOLOGICAL INFORMATION

12.1 Toxicity

Toxicity to daphnia and other aquatic invertebrates

EC50 - Daphnia - > 980 mg/l - 48 h



Toxicity to algae EC50 - Algae - 397 mg/l - 72 h NOEC - Algae - 100 mg/l - 72 h

12.2 Persistence and degradability

Biodegradability Result: - Readily biodegradable. (OECD Test Guideline 301F)

12.3 Bioaccumulative potential

No bioaccumulation is to be expected (log Pow <= 4).

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects

No data available

SECTION 13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging

Dispose of as unused product

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 datasheet 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.



SAFETY DATA SHEET

Version: 1.0 Date written: 2 Dec 2015

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

UNDERTAKING

Product Name 10x acetyl esterase reaction buffer (500mM sodium acetate pH 5.5)

Product Catalogue Name LZ-ACASE-BUFFX10

Company: Ludger Ltd

Culham Science Centre

Abingdon Oxfordshire OX14 3EB 01865 408554

Telephone: 01865 408554
Emergency Telephone: 01865 408554
Email: info@ludger.com

SECTION 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008. This substance is not classified as dangerous according to Directive 67/548/EEC.

2.2 Label elements

The product does not need to be labelled in accordance with EC directives or respective national laws.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3. 1 Substances

Formula: C₂H₃NaO₂

Molecular weight: 82.03 g/mol

CAS-No.: 127-09-3 EC-No.: 204-823-8

No components need to be disclosed according to the applicable regulations.

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact



Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Sodium oxides, Carbon oxides

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust. For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

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.

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Storage class (TRGS 510): Non Combustible Solids



7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls

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

Personal protective equipment

Eye/face protection

Safety glasses with side-shields conforming to EN166 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. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Body Protection

Impervious clothing. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

Do not let product enter drains.

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 Hq 5.5 at 25 °C Melting point/freezingpoint No data available Initial boiling point andboiling range No data available Flash point Not applicable No data available Evaporation rate Flammability (solid, gas) 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



Water solubility
Partition coefficient: n-octanol/water

Auto-ignition temperature Decomposition temperature

Viscosity

Explosive properties Oxidizing properties No data available No data available

9.2 Other safety information

No data available

SECTION 10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

No data available

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

Other decomposition products - No data available

In the event of fire: see section 5

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

No data available

Skin corrosion/irritation

No data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitisation

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

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

Reproductive toxicity

No data available



Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

Additional Information

RTECS: Not available

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

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

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects

No data available

SECTION 13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging

Dispose of as unused product

SECTION 14. TRANSPORT INFORMATION

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14.2 UN Proper Shipping Name

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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

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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

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.