Product Guide for LudgerZyme™ PNGase L Release

Product # LZ-PNGaseL-50

Ludger Document # LZ-PNGaseL-50 -Guide-v.1.0

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Specifications for LZ-PNGaseL-50

Application

LudgerZyme Peptide N-glycosidase L (PNGase L) is suitable for release of N-linked glycans in solution, and from immobilized samples. The enzyme cleaves between the innermost GlcNAc of the oligosaccharide moiety at its attachment point to the asparagine residue on the protein and subsequently converts the asparagine into aspartic acid. Released glycans with free reducing terminus can be labelled using LudgerTag labelling technology for fluorescence and high MS sensitivity detection.

Description

LudgerZyme PNGase L is a recombinant glycoamidase cloned from *Flavobacterium akiainvivens*. The enzyme is supplied glycerol free (for optimal performance in HPLC intensive methods) along with Reaction Buffer, Denaturation Solution and NP-40 Solution for efficient de-glycosylation. The methods described in this document have been developed and validated at Ludger.

Specificity

PNGase L is suitable for release of all types (high-mannose, hybrid and complex) N-glycans from glycoproteins and glycopeptides, including those from non-mammalian sources such as plants, insects and parasites carrying α1-3 linked core fucose.

Number of Samples

The enzyme is sufficient for approximately 50 samples.

Amount of Sample

As a guideline, up to 100 µg of glycoprotein per sample.

Suitable Samples

Glycoproteins and glycopeptides containing N-linked glycans.

Storage

Store at 4°C. Protect from sources of heat and light.

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 and contaminating enzymes. 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 (SDS's) 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.

Contents

Cat. #	Item	Quantity
LZ-PNGaseL-50	PNGase L (*Flavobacterium akiainvivens*) supplied in 20 mM citrate-phosphate 100 mM NaCl pH 6	1 vial of 0.100 mL

Additional Reagents and Equipment Required

- Pure water: resistivity above 18 MΩ-cm, particle free (>0.22 μm), TOC <10 ppb.
- Polypropylene reaction vials with caps.
- Water bath, oven or heating block with constant temperature maintenance at 37°C.
- Vortex or shaker.

Time Line for Procedure

Procedure	Approx. Time
Sample preparation	5 min
Protein denaturation	10 min
Addition of enzyme	5 min
Incubation	Approx. 1h

Method

Presented protocols are for in-solution release of N-glycans from glycoproteins/glycopeptides under denaturing and native conditions. Typical reaction conditions are demonstrated. The exact amount of enzyme and incubation times should be determined empirically for each glycoprotein and may require further optimisation.

De-glycosylation rate can be determined by analysis of remaining protein moiety using SDS-PAGE or alternatively, MS analysis of digested peptides. Released N-glycans can be analysed using chromatographic and mass spectrometric techniques in order to obtain their structural information.

Ludger sells an IgG glycoprotein standard (#GCP-IGG-100U) for use as a positive control in glycan release protocols.

Denaturing reaction conditions

For many glycoproteins, the conformation of the protein in its native form can create steric hindrance that restricts access of any PNGase L enzyme to certain glycosylation sites. For this reason we recommend denaturation of samples using SDS and DTT (which are components of the Denaturation Solution), prior to enzyme incubation to aid efficient de-glycosylation.

1. Sample preparation

Ensure that samples are free of other contaminating glycoproteins prior to N-glycan release. Use up to 100 µg of glycoprotein per replicate. Dry samples down if the volume exceeds 9 µL.

- Make up sample volume to 9 µL with ultrapure water.

2. Denaturation of the protein

- Add 1 µL of 10X Denaturation Solution to each glycoprotein sample. Close the reaction vials, vortex thoroughly and briefly centrifuge to ensure the samples are completely dissolved.
- Incubate the samples at 100°C for 10 minutes.

Cool the samples to room temperature and briefly centrifuge before proceeding to the next step.

3. Incubation

- Add 2 µL of 10X Reaction Buffer to each glycoprotein sample.
- Add 2 µL of 10% NP-40 solution.

PNGase L is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture when you have used denaturing conditions. Failure to include NP-40 into the denaturing protocol will result in loss of enzymatic activity.

- Add 4 µL of water.

- Add 2 µL of PNGase L. Close the reaction vials, mix gently and briefly centrifuge.
- Incubate the samples at 37°C for 1h.

Different glycoprotein classes, as well as heavily glycosylated proteins, may require different incubation times typically varying from 10 minutes up to 3 hours. Make sure total incubation time does not exceed 24 hours as this may lead to sample degradation.

Non-denaturing reaction conditions

If the native protein needs to be recovered from the reaction the denaturation step can be omitted but de-glycosylation may not be complete. When deglycosylating a native glycoprotein, it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

1. Sample preparation

Ensure that samples are free of other contaminating glycoproteins prior to N-glycan release. Use up to 100 µg of glycoprotein per replicate. Dry samples down if the volume exceeds 18 µL.

- Make up sample volume to 18 µL with ultrapure water.

2. Incubation

- Add 2 µL of 10X Reaction Buffer to each glycoprotein sample.
- Add 2-5 µL of PNGase L. Close the reaction vials, mix gently and briefly centrifuge.
- Incubate the samples at 37°C for 4-24h.

Analysis of released N-glycans

Released N-glycans can be analysed using chromatographic and mass spectrometric techniques. Refer to Ludger Guides for kits and protocols (https://www.ludger.com/product-catalogue/) for glycan clean-up (LC-EB10-A6 cartridges for MS applications), fluorophore labelling (for UHPLC and LC-MS analysis) and glycan permethylation (for MALDI-MS analysis).

Below is a reference trace for HILIC-UHPLC analysis of N-glycans released from a human IgG glycoprotein mix using PNGase L.

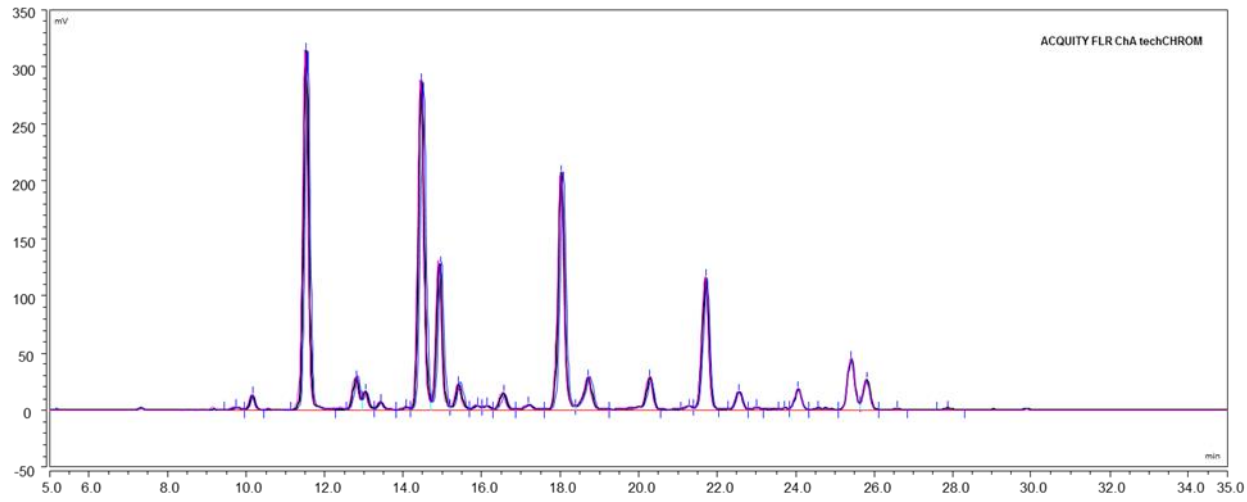

Figure 1: HILIC-UHPLC overlay of three profiles of PROC labelled N-glycans released from human IgG glycoprotein using LZ-PNGaseL-50 following 1 hour incubation protocol.

Appendix 1: Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the PNGase L for the release of glycans from glycoproteins and glycopeptides.

The positive control gives negative results.

The enzyme became inactive

Long-term storage of the PNGase L at a temperature different from that recommended can result in loss of enzymatic activity. For the best performance, store at 4°C.

Following the protein denaturation step ensure that the sample is cooled to room temperature before addition of the enzyme. Adding the enzyme to solution which has not been cooled down completely may cause enzyme denaturation and a decrease in release efficiency.

Post-release sample processing resulted in glycan loss

Make sure that your post-release glycan processing (including glycan clean-up methods) did not result in glycan loss or precipitation. For glycan preparation for chromatography and mass spectrometric applications refer to Ludger Guides (https://www.ludger.com/product-catalogue/)

The glycan release was not efficient.

The glycoproteins are not dissolved

If the solubilisation of glycoproteins is insufficient the glycan release will be incomplete. To ensure sample is

dissolved properly, vortex sample longer or make up the release solution in a larger volume of reaction mixture.

The sample contained contaminants that interfered with PNGase L activity

Please ensure that the glycoprotein solution is free from contaminants before glycan release. Avoid high ionic strength buffers in your sample as they can alter pH of the reaction mixture. Keep the pH of final reaction mixture within the PNGase L activity range (pH 6-7.5).

The incubation condition was incorrect

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 glycoprotein material than was originally estimated

Please ensure sufficient amount of sample is used.

Skewing of the results was observed.

PNGase L incubation time was not sufficient

Some glycoforms or glycosylation sites of the protein can be less prone to de-glycosylation with PNGase L. For these glycoforms and sites, glycan release can occur at a lower rate. Ensure that de-glycosylation time has been adjusted to your specific glycoprotein and its glycosylation level. Note that release will typically take longer under non-denaturing conditions.

Reagents were added in inadequate proportions

Ensure that appropriate proportion of reagents was used in the reaction. Failure in addition of Denaturation Solution may result in higher rate of sialylated glycans over neutrals being released, however, excessive amount of SDS will greatly impact enzymatic activity. Ensure that NP-40 (which stabilises the enzyme in the presence of denaturant) is present in the reaction mixture during PNGase L incubation under denaturing conditions.

Sample contains contaminating glycoproteins

PNGase L enzyme will remove N-glycans from all the proteins present in the reaction mixture. If you are interested in a specific glycoprotein, ensure that effective purification methods have been applied. Protein purity can be determined using SDS-PAGE analysis.

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-PNGaseL-50, version v1.0

SAFETY DATA SHEET

Version: 1.0
Date written: 21st July 2022

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

Product Name	**LudgerZyme Peptide N-glycosidase L (Flavobacterium akiainvivens) supplied in 20mM Citrate-Phosphate Buffer pH 6.0 with 100mM NaCl**
Product Catalogue Name	**LZ-PNGFaseL-Bulk, LZ-PNGaseL-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.

2.2 Label elements
Not a hazardous substance or mixture.

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 phosphate (anhydrous) Disodium hydrogen phosphate
Formula:	Na2HPO4
Molecular weight:	141.96 g/mol
CAS-No.:	7558-79-4
EC-No.:	231-448-7

Synonyms	Citric Acid
Formula:	HOC(COOH)(CH2COOH)2
Molecular weight:	192.12 g/mol
CAS-No.:	77-92-9
EC-No.:	201-069-1

Synonyms	Sodium chloride
Formula:	NaCl
Molecular weight:	58.44 g/mol
CAS-No.:	7647-14-5

EC-No.: 231-598-3

Synonyms Peptide N-glycosidase L (Flavobacterium akiainvivens)
Molecular weight: 50kDal
CAS-No.: -
EC-No.: -

Component	Concentration
Name: disodium hydrogen phosphate	<1%
CAS-No. 7558-79-4	
EC-No. 231-448-7	
2nd Name: citric acid	<1%
CAS-No. 77-92-9	
EC-No. 201-069-1	
Index-No.	
3rd Name: sodium chloride	<1%
CAS-No. 7647-14-5	
EC-No. 231-598-3	
Index-No.	
4th Name: Peptide N-glycosidase L (Flavobacterium akiainvivens)	<0.1%
CAS-No. 7647-14-5	
EC-No. 231-598-3	
Index-No.	

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.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

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

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
Carbon oxides, Hydrogen chloride gas, Sodium 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
Avoid dust formation. Avoid breathing vapours, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions
No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up
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
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.

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
General industrial hygiene practice.

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 Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
Contact the supplier of the EC approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
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
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
No special environmental precautions required.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance Form	Liquid, clear
Odour	No data available
Odour Threshold	No data available
pH	6.0 at 25 °C
Melting point/freezingpoint	No data available
Initial boiling point andboiling range	No data available
Flash point	No data available
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

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, Hydrogen chloride gas, Sodium oxides
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.

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
For this product a chemical safety assessment was not carried out

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.