

### Product # LC-A-24

Ludger Document # LC-A-24-Guide-v1.0

#### Ludger Ltd

Culham Science Centre Oxford OX14 3EB United Kingdom

Tel: +44 1865 408 554 Email: info@ludger.com www.ludger.com

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### Specifications for LudgerClean<sup>™</sup> A Cartridges

Application	The cartridges contain a unique solid phase extraction (SPE) resin that binds a wide range of glycopeptides and allows the enrichment of these from peptide/glycopeptide mixtures. They are compatible with many leading vacuum manifold SPE handling systems.
Description	For enrichment of glycopeptides after protease digestion (eg. Sequence grade trypsin, pronase). Suitable for enrichment/cleanup after glycopeptide labeling with AQC (6-aminoquinolyI-N-hydroxy-succinimidyl carbamate) and V-tag.
Binding Capacity	Each cartridge can typically bind glycopeptides released from up to 100 $\mu$ g of glycoprotein.
Number of Samples	LudgerClean <sup>™</sup> A cartridges are designed for single use only.
Suitable Samples	A wide range of glycopeptides can be purified. This includes the purification of glycopeptides that have been generated from different proteases, like trypsin or pronase.
Storage:	Store at room temperature in the dark. Protect from sources of heat, light, and moisture. The cartridges are stable for at least two years as supplied.
Shipping:	The product can be shipped at ambient temperature.
Handling:	Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate.
Safety:	Please read the Material Safety Data Sheets (MSDS's) for all chemicals used. All processes involving hazardous reagents should be performed using appropriate personal safety protection - eyeglasses, chemically resistant gloves (e.g. nitrile), etc and where appropriate in a laboratory fume cupboard
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For research use only. Not for human or drug use



### Additional Reagents and Equipment Required

#### Reagents

- Pure water (HPLC grade)
- Acetonitrile (HPLC grade)
- Trifluoroacetic Acid

#### Equipment

- Manifold Cartridge Holder Plate (LP-HOLDER-96)
- Plug Pack, 12 strips of 8 plugs, to seal empty cartridges in preparation for vacuum (LP-PLUG-96)
- Deep-well collection plate (Collection Plate Pack, LP-COLLPLATE-2ML-96)
- Pipettes
- Vacuum manifold

### Introduction

LudgerClean<sup>™</sup> A cartridges have been designed for enrichment of glycopeptides after protease digestion, before or after fluorophore labeling. In this specific product guide, we detail the steps required to enrich glycopeptides straight after protease digestion, without any fluorophore labelling of the glycopeptides. The cartridges are designed for use with vacuum manifolds or liquid handling robots equipped with a vacuum manifold.

### Timeline for Cleanup

The LudgerClean<sup>™</sup> A glycopeptide enrichment procedure takes approximately 90 minutes to complete.

Procedure		Time
1.	Preparation of the required solutions	30 min
	Assemble the vacuum manifold	02 min
2.	Preparation of the LC-A cartridges	10 min
3.	Preparation of samples for cleanup	10 min
	Apply the sample to A cartridges	08 min
4.	Washing the cartridges	15 min
5.	Elution of enriched glycopeptides	15 min

#### **Total Time**

90 min

# LudgerClean<sup>™</sup> A Post-digest Sample Clean-up and Enrichment

Post-digest sample **clean-up and enrichment** (to remove enzymes and peptides) is necessary for certain applications - e.g. subsequent analysis or separation by HPLC or MS - in order to analyse and investigate glycopeptides with or without further sample modification. This sample clean-up/enrichment can be achieved using the **LudgerClean<sup>TM</sup> A** cartridges which are supplied in the kit (Cat # LC-A-24).

LudgerClean A cartridges are designed for use with any standard 96 well micro-titre plate compatible vacuum manifold (E.g. Ludger-Velocity Vacuum Manifold System - LC-VAC-MANIFOLD-KIT) or a liquid handling robot. In order to use these cartridges with a vacuum manifold, the user will also need a Cartridge Holder Plate (LP-HOLDER-96) and a set of plugs to fill any holes in the plate that are not used, (Plug Pack, LP-PLUG-96)

NOTE: The devices can be used without a vacuum manifold. In this case a pipette can be used to help push the sample through the cartridge by use of air displacement if gravity alone takes longer than 5 minutes. It is important to ensure that the contact times for the binding, washing and elution steps are consistent and gradual. Fast times may result in poor binding and selective elution. If a solution passing through a cartridge is slower than 5 minutes then use a higher vacuum pressure or pipette to speed up the process, allowing at least 1 minute solvent contact time with the cartridge.

#### Step 1: Prepare the washing solutions

Preparation of solutions 1, 2, and 3 can be done using fleshly opened pure TFA (analytical grade) directly or preparing beforehand a 10% TFA solution. To prepare a 10% TFA solution measure 9 mL of water and add 1 mL of TFA; the remaining 10% TFA solution can be stored in the freezer for future use; acetonitrile and water (18.2 MΩ·cm) are also required.

**NOTE FOR SOLUTIONS 2 and 3**: These solutions should be prepared by measuring the volumes of water, acetonitrile and TFA independently and accurately before combining together. The composition of these solutions is critical for good enrichment results.

Solution 1. 0.1 % TFA. For preparation of 100 mL do the following:

Add 1mL of the 10% TFA solution to 99 mL of water.

Or add 100  $\mu I$  of pure TFA to 99.9 mL of water.

- Solution 2. **76 % acetonitrile (ACN)**, **0.1 % TFA**. For preparation of 200 mL do the following: Mix together: 152 mL of ACN, 46 mL of water and 2 mL of the 10% TFA solution. Or 152 mL of ACN, 47.8 mL of water and 200 μL of pure TFA.
- Solution 3. **40 % ACN**, **0.1 % TFA**. For preparation of 100 mL do the following: Mix together: 40 mL of ACN, 59 mL of water and 1 mL of the 10% TFA solution. Or 40 mL of ACN, 59.9 mL of water and 100 µL of pure TFA

Once the washing solutions are prepared and used, the spare solution can be store in the fridge and re-used.

### Instructions for use of the LC-A cartridges with a vacuum manifold

#### **Step 2: Prepare the LC-A cartridges**

• Place a LudgerClean<sup>™</sup> A cartridge for each sample into the cartridge holder, and position onto a vacuum manifold.

For a more in-depth description of how to set up the cartridges on the vacuum manifold see the Ludger-Velocity SPE vacuum manifold system Guide found on our website <u>www.ludger.com</u>

• Prime each LudgerClean<sup>™</sup> A cartridge by adding the following solutions, applying a slow vacuum to drain and discarding the flow-through:

1 <sup>st</sup> wash: Solution 1 (0.1% TFA in water)	-	1 mL
2 <sup>nd</sup> wash: Solution 2 (76% acetonitrile, 0.1% TFA)	-	1 mL

#### Step 3: Prepare the protease digested samples and apply to the LC-A cartridges

 Pipette 150 µL of 100% acetonitrile into the protease digested sample (typically the volume of sample is 15-25 µL). Gently mix the sample by pipette action and immediately load the sample onto a primed cartridge. Repeat with all the protease digested samples. Wait 5 minutes and then apply a slow vacuum (taking approximately one minute) to drain the LC-A cartridge.

Note: In order to avoid sample precipitation, the addition of acetonitrile should be performed just before applying the sample onto the cartridge.

#### Step 4: Wash the LC-A cartridges

• Wash the cartridges with 3 x 1 mL of solution 2 (76% acetonitrile, 0.1% TFA) and discard the flow-through.

#### **Step 5: Elute the glycopeptides**

- Remove the top of the vacuum manifold holding the cartridge holder, place a deep-well collection plate in the vacuum manifold and re-place the manifold top, with the cartridge holder, back in place on top of the manifold.
- Elute the glycopeptides by adding 0.5 mL of solution 3 (40% acetonitrile, 0.1% TFA) to each cartridge and wait for 5 minutes. Apply a slow vacuum taking approximately one minute to completely elute the samples.

#### The samples are now ready for LC/MS and MALDI analysis.

When a weaker than recommended concentration is used, we recommend concentrating the sample using centrifugal evaporation or the equivalent.



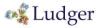
### Warranties and Liabilities

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

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

### **Document Revision Number**

Document # LC-A-24-guide for glycopeptide enrichment-v1.0



### SAFETY DATA SHEET

Version: 1.1 Date reviewed: 12<sup>th</sup> Mar 2021

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

#### / UNDERTAKING

Product Name	LudgerClean™ A cartridges
Product Catalogue Name	LC-A-24
Company:	Ludger Ltd Culham Science Centre Abingdon Oxfordshire OX14 3EB
Telephone: Emergency Telephone: Email:	01865 408554 01865 408554 info@ludger.com

#### **SECTION 2. HAZARDS IDENTIFICATION**

#### 2.1 Classification of the substance or mixture

This product is not classified as dangerous according to Regulation (EC) No. 1272/2008

#### 2.2 Label elements

This product is not classified as dangerous according to Regulation (EC) No. 1272/2008

#### Hazard Statement(s)

No data available.

#### Precautionary Statement(s)

No data available.

#### 2.3 Other hazard information:

No data available.

#### **SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS**

#### 3.1 Substances

Synonyms: Formula: Synthetic amorphous silica gel Information not available

Component		Concentration	
Name	A cartridge resin	>95%	
CAS-No.	None		
EC-No.	None		

#### **SECTION 4. FIRST AID MEASURES**

#### 4.1 Description of first aid measures

#### **General Advice**

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

#### If Ingested

Rinse mouth with plenty of water if the person is conscious. Never give anything by mouth to an unconscious person. Drink plenty of fluids afterwards.



#### If skin is exposed

Wash effected area(s) with plenty of soap and water.

#### If eyes are exposed

Flush eyes with plenty of water/ eye wash, making sure that the eye is rinsed well, paying attention to the areas around the eyelids.

#### If inhaled

Move person into fresh air. If breathing has stopped give artificial respiration.

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

No data available

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

No data available

#### SECTION 5. FIRE-FIGHTING MEASURES

#### 5.1 Extinguishing media

Use a dry chemical, CO<sub>2</sub>, water spray or alcohol foam media. Choose and extinguisher which is appropriate for the surrounding conditions.

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

No data available.

#### 5.3 Advice for firefighters

For extreme fires, wear self-contained breathing apparatus for firefighting.

#### SECTION 6. ACCIDENTAL RELEASE MEASURES

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

Ventilate the affected area thoroughly and shut off any sources of ignition. Use PPE described in Section 8. Avoid causing dust when sweeping up the chemical. Avoid breathing in the dust.

#### 6.2 Environmental Precautions

Do not let the chemical enter that drainage system.

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

Collect the spilt chemical, creating as little dust as possible. Sweep up the chemical and shovel into a suitable container with and air tight lid. Arrange collection on the waste material.

#### 6.4 Reference to other sections

Section 8 and 13.

#### **SECTION 7. HANDLING AND STORAGE**

#### 7.1 Precautions for safe handling

To handle/ work with the product in a well-ventilated area and user to wear PPE.

#### 7.2 Conditions for safe storage

Keep the products in a dry and well ventilated storage cupboard/cabinet, in original packaging or in a container with a lid. Keep product away from direct sunlight. Store at Room temperature.

#### 7.3 Specific end uses

No data available.

#### SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

#### 8.1 Control parameters

This product contains no substances with occupational exposure limit values.



#### 8.2 Exposure controls

#### Appropriate engineering controls

General advice to the user is to wear PPE, and wash hands, avoid contact with skin. To follow good laboratory practice for safety and hygiene.

#### **Personal Protective Equipment**

#### Eye / face protection

Safety glasses with side shields conforming to EN 166. Use eye equipment for eye protection tested and approved under appropriated government standards such as NIOSH (US) or EN 166 (EU).

#### Skin protection

Wear gloves when handling the product. Gloves must conform to the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it. Inspect gloves before use for tears and holes, gloves to be removed using the proper removal technique (without touching the outer surface of the glove) to avoid skin contact with the product. To be disposed of as chemical waste.

#### **Body Protection**

Laboratory over cover such as a laboratory coat or any other similar coverings.

#### **Respiratory protection**

Product to be used under extraction or well-ventilated area, no further protection is required for the amount per cartridge.

Form: Powder

#### **Thermal hazards**

No data available.

#### **SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES**

#### 9.1 Information on basic physical and chemical properties

#### Appearance

Colour: White
None
None
No data available
Insoluble
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 under the recommended storage conditions in Section 7.

#### **10.3 Possibility of hazardous reactions**

No data available

#### **10.4 Conditions to Avoid**

Extreme temperatures, High or low.

#### **10.5 Incompatible materials**

Strong oxidising agents, strong acids and hydrogen fluoride.

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

Formed under fire/ high temperatures – Silicon oxides.

#### **SECTION 11. TOXICOLOGICAL INFORMATION**

#### 11.1 Information on toxicological effects

To the best of our knowledge, the toxicological properties of this product have not been fully investigated.

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 No data available

#### 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

This product has no known adverse effect on human health.

Inhalation	May cause respiratory tract irritation.
Ingestion	No data available
Skin	May cause skin irritation.
Eyes	May cause eye irritation.

#### Signs and symptoms of exposure No data available



#### **SECTION 12. ECOLOGICAL INFORMATION**

The eco-toxicological properties of this material have not been fully investigated. **12.1 Toxicity** No data available.

#### 12.2 Persistence and degradability

No data available.

#### 12.3 Bio-accumulative potential

Does not bio-accumulate.

#### 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 waste disposal service to dispose of waste product. Do not send as general waste for disposal.

#### Contaminated packaging

Dispose of as unused product, follow the above advice.

#### SECTION 14. TRANSPORT INFORMATION

<b>14.1 UN Num</b> ADR/RID: -	nber IMDG: -	IATA: -	
<b>14.2 UN Prop</b> ADR/RID: IMDG: IATA:	<b>ber Shipping Name</b> Not classed as dangerous goods Not classed as dangerous goods Not classed as dangerous goods		
14.3 Transpo ADR/RID: -	ort hazard class(es) IMDG: -	IATA: -	
14.4 Packing ADR/RID: -	<b>i group</b> IMDG: -	IATA: -	
<b>14.5 Environ</b> ADR/RID: No	mental hazards IMDG Marine pollutant: No	IATA: No	
14.6 Special precautions for user			

#### 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.