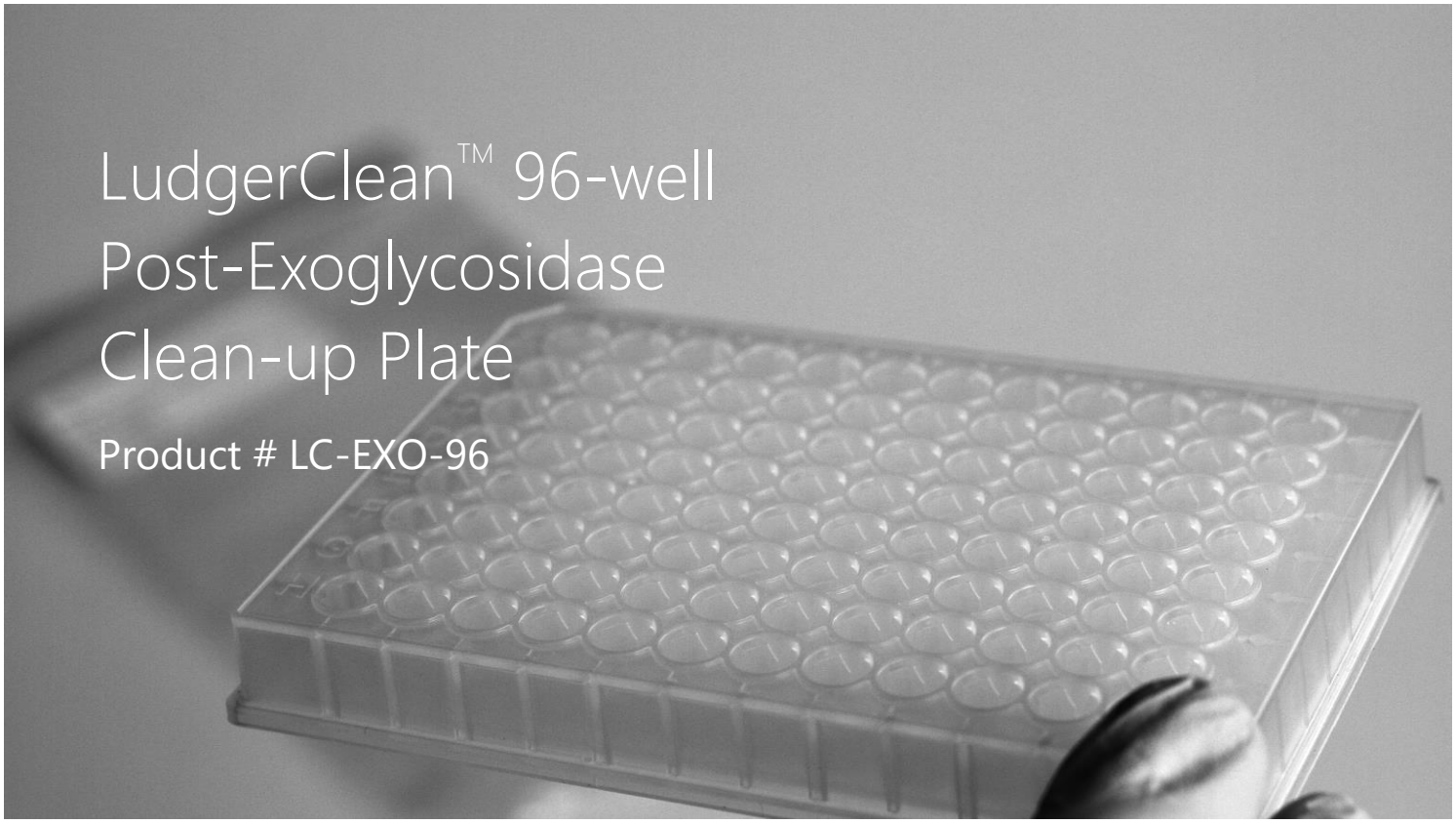

LudgerClean™ 96-well Post-Exoglycosidase Clean-up Plate

Product # LC-EXO-96

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

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Contents

LudgerClean Post-Exoglycosidase Clean-up Plate

Applications	**Post-exoglycosidase Clean-up** For removal of exoglycosidase enzymes and other protein material following glycan enzymatic digestion/ sequencing. This will prevent contamination of HPLC columns during subsequent chromatographic analysis. The plate can also be used to remove exoglycosidases or other proteins before mass spectrometry analysis of glycans.
Description	The LC-EXO-96 plate is a 96 well membrane-bottom plate containing a specialized modified polyethersulfone membrane with a molecular weight cut-off of approximately 30 kDa. This product is compatible with negative pressure systems (like most of popular vacuum manifold systems, including the model supplied by Ludger) or centrifuges equipped with 96-well format plate rotor. Glycans pass through the membrane whilst proteins are retained on the membrane allowing separation of these two components.
Number of Samples	Sufficient for up to 96 samples.
Amount of Sample	Up to 350 µL per well
Centrifugal Force	1500 x g
Operating Vacuum	10-20 in Hg (approx. 0.35-0.7 bar)
Suitable Samples	Unlabelled and fluorophore labelled (e.g. 2-AB, 2-AA or procainamide labelled) glycans released from glycoproteins or other sources and treated with exoglycosidase enzymes.
Storage	Store at room temperature. Protect from sources of heat, light, and moisture. When stored correctly, the products should be stable for 36 months from date of purchase.
Shipping	The product should be shipped at ambient temperature.

For research use only. Not for human or drug use

Kit Contents

The kit contains the following materials:

Cat. #	Item	Quantity
LC-EXO-96	LudgerClean 96-well Post-Exoglycosidase Clean-up Plate	1

Additional Reagents and Equipment Required

For a full list of vacuum manifold accessories see the Ludger-Velocity-Guide available from our website or upon request.

- Pure water: resistivity above 18 MΩ-cm, particle free (>0.22 µm), TOC <10 ppb
- Vacuum manifold and trap suitable for 96 well format SPE plates (cat. no. LC-VAC-MANIFOLD-KIT and LC-VACUUM-TRAP-KIT) OR centrifuge equipped with 96-well format plate rotor
- Short (1.2 mL) or deep-well (2 mL) collection plate for collecting glycans (cat. No. LP-COLLPLATE-2ML-96).
- Collection plate lid (cat. No. LP-COLLPLATE-LID-96) – optional.

Safety and Handling

- Ensure that any glass, plasticware 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.
- Once used, the plate should be discarded according to local safety rules.

Clean-up Procedure

Time Line for Procedure

Procedure	Time
Sample loading	10 minutes
Elution of glycans	40 minutes
Drying glycans	as required
Total time	50 minutes plus drying time

Method

Clean-up can be performed by using either any compatible negative pressure system or centrifugation. Below protocol is for post-exoglycosidase clean-up using Ludger vacuum manifold system. **In case centrifugation is used, vacuum steps can be replaced with 20 min centrifugation steps at 1500 x g speed.**

Apply the samples onto the clean-up plate

- Place a 96-well collection plate (LP-COLLPLATE-2ML) inside the vacuum manifold. Assemble the manifold with the post-exoglycosidase clean-up plate on top ensuring that the collection plate is in-line with the wells (if using centrifugation instead, place the clean-up plate directly on top of the collection plate).
  Ensure that the distance between the collection plate and the manifold top is as small as possible to reduce the gap between the clean-up plate and the collection plate and prevent sample cross-contamination.
- **Pipette the glycan samples into the post-exoglycosidase clean-up plate wells. Wash out each sample vial with 100 µL of water and add this to the clean-up plate wells**. Apply a vacuum and adjust to between -0.3 and -0.5 bar until the liquid has all gone through the wells. Open the tap to release the vacuum.
  The maximum pressure used should be no more than -0.7 bar.

Wash the wells of the clean-up plate

- **Pipette 100 µL of water into each well to wash through any remaining sample**. Apply a vacuum and adjust to between -0.3 and -0.5 bar until the liquid has all gone through the wells. Open the tap to release the vacuum.

Dry the glycans if necessary

- At this stage glycan samples may be at sufficient concentration for their intended use. Alternatively you additionally concentrate the glycans in a vacuum centrifuge. We do not recommend applying heat at this stage. Long drying times under elevated temperature may lead to glycan desialylation.

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-EXO-96-guide, version v1.0

Appendix 1: Troubleshooting Guide

1. **Liquid does not flow.**
    - Insufficient vacuum/ centrifugation time: We recommend using extended vacuum/ centrifugation time for samples where high protein concentration/ high sample density is expected. Do not use higher than recommended vacuum settings/ centrifugation speed as this may disrupt the membrane structural integrity.
    - Too much protein material: High concentration of protein or other high molecular weight material present in the sample can disrupt the liquid flow through the membrane. If liquid does not continue to pass despite extended vacuum/ centrifugation time, we recommend splitting the sample into two or more replicates. Maximum amount of protein per well should not exceed 20 µg.

Appendix 2: Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620 Email: info@ludger.com, Website: www.ludger.com
Identification of the substance	LudgerClean Post-Exoglycosidase Clean-up Plates
Composition	Plate of polypropylene containing modified polyethersulfone membrane.
Hazard indentification	Non hazardous.
Fire fighting measures	Non hazardous. Water spray or appropriate foam according to surrounding fire conditions.
Accidental release measures	Not applicable.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Constructed of solid plastic and polymeric materials.
Stability and reactivity	Not combustible.
Toxilogical information	Toxicological, carcinogenic and mutagenic properties have not been investigated.
Ecological information	Data not available.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Contact Ludger Ltd for transportation information.
Regulatory information	Data not available.
Other information	**The advice offered 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.**