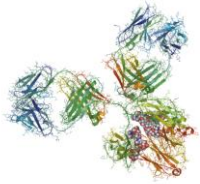

Ludger

Product Guide for Ludger Clean™ Pre-Permethylation Clean-up Plate

Ludger Product Guide: LC-PERMET-96

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Enrichment of glycans prior to Permethylation

(Specifications for LC-PERMET-96 plate)

Application The 96 well plate is used for removal of excess salts, detergent, proteins and other impurities after the release of N-glycans from glycoproteins. This clean-up plate is used to enrich N-glycans prior to performing permethylation of released glycans.

Description The LC-PERMET-96 plate is a 96 well membrane-bottom plate containing a specialized glycan binding membrane. This product is designed for use with both the vacuum manifold that can be purchased from Ludger or with other popular vacuum manifold systems. Impurities from enzymatic N-glycan release can be washed through the membrane whilst glycans are bound to the membrane. The enriched glycans can be eluted in the end and dried down before permethylation.

Number of Samples Sufficient for up to 96 samples.

Volume of Samples Up to 350 µL per well.

Suitable Samples Any unlabelled glycans or glycans released from glycoproteins by PNGaseF.

Storage Store at room temperature. Protect from sources of heat, light, and moisture. When stored correctly, the plate 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:

Catalogue Id.	Item	Quantity
LC-PERMET-96	LudgerClean 96 well Pre-Permethylation Clean-up plate	1 Plate

Additional Reagents and Equipment Required

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

- Pure water: resistivity above 18 MΩ-cm, particle free (>0.22 µm), TOC <10 ppb
- 70 % ethanol solution
- Acetonitrile (HPLC grade)
- 1% Formic acid
- Vacuum manifold suitable for 96 well format SPE plates (cat. no. LC-VAC-MANIFOLD Kit)
- Vacuum trap (cat. No. LC-VACUUM-TRAP-KIT)
- 2 mL collection plate for collecting glycans (cat. No. LP-COLLPLATE-2ML-96).
- 1.5 mL Eppendorf vials for drying purified glycans.
- Collection plate lid (optional) (cat. No. LP-COLLPLATE-LID-96).

Safety and 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.
- Once used, the plate should be discarded according to local safety rules.

Procedure Time Line for 96 samples

Procedure	Time
Assemble the vacuum manifold	05 min
Priming / Preparation of clean-up plate	30 min
Adding sample	10 min
Eluting glycans	30 min
Drying glycans	As required
Total time	1 hour 15 min plus drying time

Clean-up procedure

1 Assemble the vacuum manifold

Either follow your usual procedure for assembling your current vacuum manifold system or follow the instructions supplied with a Ludger vacuum manifold system (LC-VAC-MANIFOLD-KIT – see Ludger-Velocity-Guide for kit manual). Place a collection plate or a suitable container inside the manifold to collect waste (an empty pipette tip box usually fits). Place the top back on the manifold and place the pre-permethylation clean-up plate on top of the manifold.

2 Priming / Preparation of the clean-up plate

Pipette 200 µL of a 70 % ethanol solution into the plate wells that are to be used to wet the membrane. Apply a vacuum and adjust to between -0.05 and -0.2 bar until all the ethanol solution has all gone through the wells. Open the tap to release the vacuum. Pipette 200 µL of water into each well to wash the membrane. Apply a vacuum and adjust to between -0.05 and -0.2 bar until the water has all gone through the wells. Pipette 200 µL of acetonitrile into each well to prime the membrane. Apply a vacuum and adjust to between -0.05 and -0.2 bar until the acetonitrile has all gone through the wells.

When applying the vacuum, you may have to push the base plate down onto the manifold until the vacuum takes hold. **The maximum pressure used should be no more than -0.5 bar**

3 Preparation of the released N-glycan samples

Pipette 280 µL of 100% acetonitrile into each sample and gently mix using pipetting action.

4 Apply the samples to the plate

Load each sample into a primed wells of the plate. Without applying a vacuum allow the sample to settle into the plate well for 5 minutes. Apply a vacuum and adjust to between -0.05 and -0.1 bar until the acetonitrile sample solution has all gone through the wells. Discard the waste.

5 Wash off non-glycan contaminants

Add 200 µL of acetonitrile to each well containing sample. Apply a vacuum and adjust to between -0.05 and -0.1 bar until the acetonitrile has all gone through the wells. Repeat with two additional washes of 200 µL of acetonitrile. Discard the waste.

6 Elute the enriched glycans

Place a 96 well collection plate (LP-COLLPLATE-2ML) inside the vacuum manifold. Assemble the manifold with the pre-permethylation clean-up plate on top ensuring that the collection plate is in-line with the wells. 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 (spacers may be required to lift the collection plate).

Add 100 µL of 1% formic acid to each well containing sample. Apply a vacuum and adjust to between -0.05 and -0.1 bar until the liquid has all gone through the wells. Open the tap to release the vacuum. Apply a higher vacuum (-0.5 bar) to remove as much remaining water from the wells (and underneath the membrane) as possible. Open the tap to release the vacuum. Repeat the higher vacuum (-0.5 bar) step for a second time.

Add another 100 µL of 1% formic acid into each well to wash through any remaining released N-glycans in each sample well. Apply a vacuum and adjust to between -0.05 and -0.1 bar until the liquid has all gone through the wells. Open the tap to release the vacuum. Apply a higher vacuum (-0.5 bar) to remove as much remaining 1% formic acid from the wells (and underneath the membrane) as possible. Open the tap to release the vacuum. Repeat the higher vacuum (-0.5 bar) step two additional times. These vacuum steps are done to remove as much remaining liquid from the wells (and underneath the membrane) as possible.

7 Transfer and dry down the samples

Samples should be transferred to 1.5 mL Eppendorf vials and the samples should down completely using a centrifugal evaporator (approximately 8 hours). We do not recommend applying heat at this stage. Only use a good quality vacuum centrifuge as long drying times or heat 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

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Appendix 1: Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the LudgerClean pre-permethylation clean-up plate.

a) Liquid does not flow.

The membrane requires pre-wetting with ethanol otherwise aqueous solutions will not flow through the membrane.