



**Ludger Clean<sup>TM</sup>**

# **Post-Exoglycosidase Clean-up Plate**

**Product Code LC-PBM-96**

## **Product Guide**

**Version 1.1**

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## Ludger Clean Post Exoglycosidase Cleanup Plate

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<b>Application</b>	For removal of exoglycosidase enzymes after glycan 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.
<b>Description</b>	The LC-PBM-96 plate is a 96 well membrane-bottom plate containing a specialized protein binding membrane with a nominal pore size of 0.45 µm. <b>This product is designed for use with a vacuum manifold system, which can either be purchased from Ludger but the device is also compatible with most popular vacuum systems.</b> Glycans pass through the membrane whilst proteins are bound to the membrane allowing separation of these two components.
<b>Number of Samples</b>	Sufficient for up to 96 samples.
<b>Amount of Sample</b>	Up to 350 µL per well
<b>Suitable Samples</b>	2-AB, 2-AA labelled or unlabelled glycans from glycoproteins.
<b>Storage</b>	Store at room temperature. Protect from sources of heat, light, and moisture. When stored correctly, the reagents should be stable for 36 months from date of purchase.
<b>Shipping</b>	The product should be shipped at ambient temperature.

**For research use only. Not for human or drug use**

## Kit Contents

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The kit contains the following materials and reagents:

Cat. #	Item	Quantity
LC-PBM-96	LudgerClean 96 Protein Binding Membrane Plate	1
	Plate Lid	1

## Additional Reagents and Equipment Required

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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 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).
- Collection plate lid (optional). (cat. No. LP-COLLPLATE-LID-96).

## Safety and Handling

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- 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.

## Time Line for Procedure

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<b>Procedure</b>	<b>Approx Time</b>
<b>1. Assemble the vacuum manifold</b>	<b>5min</b>
<b>2. Preparation of PBM plate</b>	<b>5min</b>
<b>3. Binding sample</b>	<b>1h</b>
<b>4. Eluting glycans</b>	<b>5 min</b>
<b>5. Drying glycans</b>	<b>as required</b>
 <b>Total time</b>	 <b>1 hour 15 min plus drying time</b>

## Method

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### 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).

Put a collection plate or other suitable container inside the manifold to collect waste (an empty pipette tip box usually fits).

Put the top back on the manifold.

Place a protein binding plate on top of the manifold.

### 2 Preparation of Protein Binding Plate

Pipette 100  $\mu$ l of methanol into the plate wells that are to be used (to wet the membrane).

Apply a vacuum and adjust to between -3 and -5" Hg (using the Ludger LC-VAC-MANIFOLD) until the methanol has passed through the well.

Pipette 300  $\mu$ l of water into each well to wash away the methanol. The membrane will remain wet.

Repeat with 2 more 300  $\mu$ l washes of water.

Tap the plate firmly to make sure any drops are removed from the bottom of the plate

Open the tap to release the vacuum

When applying the vacuum you may have to push the base plate down onto the manifold until the vacuum takes hold.

(For the Ludger vacuum manifold use the screw adjuster on the manifold to adjust the pressure to between -3 and -5" Hg , *the maximum pressure used should be no more than 15" Hg*).

### 3 Binding Sample

Remove the plate from the manifold, place on top of a 2ml square bottomed collection plate (LP-COLLPLATE-2ML-96).

Pipette the exoglycosidase digested glycan samples into the plate wells.

Cover the plate with the lid provided.

Leave to bind for 1 hour at room temperature.

(This can be left on a tilter/shaker if available).

## 4 Elute the glycans

Place the 2 mL square bottomed collection plate (which the binding plate was on) inside the manifold.

Assemble the manifold with the protein binding plate on top ensuring that the collection plate is in-line with the wells.

Apply a vacuum and adjust to between -3 and -5" Hg until the glycan/enzyme solution has passed through the wells.

Pipette 100 µl of water into each well to wash the membrane of any remaining glycans.

Apply a vacuum and adjust to between -3 and -5" Hg until the liquid has all gone through the well.

Tap the plate firmly to make sure any drops are removed from the bottom of the plate.

Open the tap to release the vacuum.

## 5 Dry Glycans

At this stage your glycan samples may be sufficiently concentrated for their intended use, Alternatively you can dry the glycans in a vacuum centrifuge. We do not recommend applying heat at this stage, and only use a good quality vacuum centrifuge as long drying run times e.g. overnight, may lead to glycan desialylation.

## Warranties and liabilities

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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

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The following is a guide to the most likely problems associated with the use of the PBM kit for exoglycosidase removal from 2-AB labeled glycans.

### **Liquid does not flow.**

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

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## Appendix 2 – Material Safety Data Sheet

<b>Manufacturer</b>	Ludger Ltd Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620 Email: safety@ludger.com, Website: www.ludger.com
<b>Identification of the substance</b>	LudgerClean PBM plates
<b>Composition</b>	Plate of polypropylene containing protein absorption discs.
<b>Hazard identification</b>	Non hazardous.
<b>First aid measures</b>	In case of contact: Eyes: irrigate with plenty of water. Skin: wash with soap and water. Ingestion: drink plenty of water. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
<b>Fire fighting measures</b>	Non hazardous. Water spray or appropriate foam according to surrounding fire conditions.
<b>Accidental release measures</b>	Not applicable.
<b>Handling and storage</b>	Store at room temperature. Handle in accordance with Good Laboratory Practice.
<b>Exposure Controls /</b>	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
<b>Physical and chemical properties</b>	Constructed of solid plastic and polymeric materials
<b>Stability and reactivity</b>	Not combustible.
<b>Toxicological information</b>	Toxicological, carcinogenic and mutagenic properties have not been investigated.
<b>Ecological information</b>	Data not available.
<b>Disposal considerations</b>	No special requirements. Dispose of according to local requirements.
<b>Transport information</b>	Contact Ludger Ltd for transportation information.
<b>Regulatory information</b>	Data not available.
<b>Other information</b>	<b>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.</b>