Product Guide for Ludger Clean™ Post-endoglycosidase and Post-exoglycosidase Clean-up Plate

Part of the Ludger-Velocity™ Fast Glycan Analysis Range.

(Ludger Product Guide: LC-PBM-96)

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Ludger Clean Post-exoglycosidase and Post-endoglycosidase Cleanup Plate

Applications

Post-exoglycosidase Cleanup

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.

Post-endoglycosidase Cleanup

For removal of PNGase F and other protein and peptide material following the acidification of N-glycans following their release from glycoproteins. Using this protein binding membrane will provide cleaner samples and increase the signal for the subsequent fluorescent labelling of the N-glycans. The plate can also be used to remove PNGase F and other protein and peptide material before permethylation and/or mass spectrometry analysis of N-glycans.

Description

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. **This product is designed for use with both the vacuum manifold that can be purchased from Ludger or with other popular vacuum manifold systems.** Glycans pass through the membrane whilst proteins are bound to 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

Suitable Samples Unlabelled glycans from glycoproteins, fluorophore labelled glycans e.g. 2-AB or 2-AA from glycoproteins, released and acidified N-glycans from glycoproteins

Storage 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.

Shipping The product should be shipped at ambient temperature.

For research use only. Not for human or drug use

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Kit Contents

The kit contains the following materials:

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

- 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.

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Post-exoglycosidase Cleanup procedure

Time Line for Procedure

Procedure	Time
Assemble the vacuum manifold	5 min
Preparation of PBM plate	5 min
Binding sample	1 hour
Eluting glycans	5 min
Drying glycans	as required
Total time	1 hour 15 min plus drying time

Method

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). Place the top back on the manifold and place the protein binding plate on top of the manifold.

2 Preparation of Protein Binding Plate

Pipette 100 µL of methanol into the plate wells that are to be used to wet the membrane. Apply a vacuum and adjust to between -0.1 and -0.2 bar (using the Ludger LC-VAC-MANIFOLD) until the methanol has passed through the wells. Pipette 300 µL of water into each well to wash away the methanol. Apply a vacuum and adjust to between -0.1 and -0.2 bar until the water has passed through the wells. The membrane will remain wet. Repeat with two further 300 µL washes of water. Tap the plate firmly to make sure any drops are removed from the bottom of the plate and 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 -0.1 and -0.2 bar. **The maximum pressure used should be no more than -0.5 bar**

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3 Binding sample

Remove the PBM plate from the top of the manifold and place on top of a 2 ml square bottomed collection plate (LP-COLLPLATE-2ML-96). Pipette the exoglycosidase digested N-glycan samples into the plate wells and cover the plate with the lid provided. Leave to bind for 1 hour at room temperature. This can be left on a plate shaker if available.

4 Elute the glycans

Place a 96-well collection plate (LP-COLLPLATE-2ML) inside the vacuum manifold. Assemble the manifold with the protein binding 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 PBM plate and the collection plate (spacers may be required to lift the collection plate).

Apply a vacuum and adjust to between -0.1 and -0.2 bar until the glycan/enzyme solution has passed through the wells. Pipette 100 µL of water into each well to wash the membrane and elute any remaining glycans. Apply a vacuum and adjust to between -0.1 and -0.2 bar until the water has all gone through the wells. 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 the 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. Only use a good quality vacuum centrifuge as long drying times e.g. overnight, may lead to glycan desialylation.

Post-endoglycosidase Cleanup procedure

Time Line for Procedure

Procedure	Time
Assemble the vacuum manifold	5 min
Preparation of PBM plate	30 min
Adding sample	10 min
Eluting glycans	30 min
Drying glycans	8 hours
Total time	2 hour 15 min plus drying time

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Method

Prior to cleaning up N-glycans following PNGase F digestion we recommend hydrolysing the N-glycans with 1% formic acid. The addition of 1 % formic acid solution to released N-glycan samples aids in the hydrolysis of the glycosylamine form of the N-glycans following PNGase F release. Hydrolysing the glycosylamine promotes the formation of a reducing end which enables the glycans to be fluorescently labelled.

Briefly, prepare a solution of 1% formic acid by adding 50 µL formic acid to 4950 µL water. Add 20 µL of the 1 % formic acid solution to each sample, mix all the samples on a plate shaker or vortexer for 1-2 minutes to make sure that all the samples are re-dissolved and then briefly centrifuge. Incubate at room temperature for 50 minutes. Following this incubation, the samples need to be cleaned up using the Protein Binding Membrane plate straight away. Do not leave the samples in acid.

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). Place the top back on the manifold and place the protein binding plate on top of the manifold.

2 Preparation of Protein Binding Plate

Pipette 100 µL of methanol 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 methanol has all gone through the wells. Open the tap to release the vacuum. Pipette 300 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. Apply a higher vacuum (-0.5 bar) to remove as much remaining water from the wells (and underneath the membrane) as possible. Discard the waste. Remove the PBM plate from the vacuum manifold and blot the bottom of the plate on a paper towel to remove the excess water.

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**

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3 Sample clean-up

Place a 96-well collection plate (LP-COLLPLATE-2ML) inside the vacuum manifold. Assemble the manifold with the PBM 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 PBM plate and the collection plate (spacers may be required to lift the collection plate).

Pipette the released N-glycan samples in 1 % formic acid into the PBM plate wells. Wash out each sample well or vial with 100 µL of water and add this to the PBM plate wells. Apply a vacuum and adjust to between -0.05 and -0.2 bar until the liquid has all gone through the wells. Open the tap to release the vacuum. Pipette 100 µL of water into each well to wash through any remaining sample. Apply a vacuum and adjust to between -0.05 and -0.2 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.

4 Dry down the samples

Transfer the eluted N-glycan samples from the 96-well collection plate to a non-skirted 96 well PCR plate (300 µL volume) or 0.5 mL centrifuge vials. Dry the samples down completely using a rotary speed vac (approximately 8 hours). We do not recommend applying heat at this stage. Only use a good quality vacuum centrifuge as long drying times e.g. overnight, 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.

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Appendix 1 : Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the PBM kit for exoglycosidase and enodglycosidase, protein and peptide removal from unlabelled glycans, 2AB labelled glycans and released and acidified N-glycans from glycoproteins.

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

Manufacturer	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
Identification of the substance	LudgerClean PBM plates
Composition	Plate of polypropylene containing protein absorption discs.
Hazard indentification	Non hazardous.
First aid measures	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.
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.**

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