Product Guide for Ludger Clean™ Post-glycosidase Clean-up Plate

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

Product # LC-PBM-96

Ludger Document # LC-PBM-96-Guide-v3.0

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Ludger Clean Post-glycosidase Clean-up Plate

Applications

Post-exoglycosidase clean-up

For removal of exoglycosidase enzymes and other protein and peptide contaminants from unlabeled glycan mixtures. This prevents contamination of HPLC columns during subsequent chromatographic analysis. The plate can also be used to remove proteins before mass spectrometry analysis of glycans.

Post-endoglycosidase Clean-up

For removal of endoglycosidase enzymes and other protein and peptide material following enzymatic release of glycans from glycoproteins. Using this protein binding membrane will provide cleaner samples and increase the signal for the subsequent fluorescent labelling of the glycans. The plate can also be used to remove the enzymes and other protein and peptide material before permethylation and/or mass spectrometry analysis of 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 and with a centrifuge equipped with a 96-well plate rotor.** 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 Underivatised glycans released enzymatically from glycoproteins including acidified N-glycans released 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

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 and trap suitable for 96 well SPE plates (cat. no. LC-VAC-MANIFOLD-KIT and LC-VACUUM-TRAP-KIT) OR centrifuge equipped with 96-well plate rotor
- 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.

Post-exoglycosidase clean-up procedure

Timeline for Procedure

Procedure	Time
Preparation of PBM plate	5 min
Binding sample	1 hour
Eluting glycans	5 min
Drying glycans	as required
Total time	1 hour 10 min plus drying time

Method

Clean-up can be performed by using either a compatible negative pressure system or 96 well plate compatible centrifuge. Centrifugation is recommended for clean-up of samples containing foaming agents such as surfactants. The protocol shown below is for the post-exoglycosidase clean-up using the Ludger vacuum manifold system. **When centrifugation is the preferred method, the vacuum steps can be replaced with 3-minute centrifugation steps at 800 x g speed at room temperature.**

1 Preparation of the Protein Binding Plate

Assemble the vacuum manifold with a suitable waste collection container inside. Place the protein binding plate on top of the manifold.

- Pipette 100 µL of methanol into each well of the plate, that is to be used, to wet the membrane. Apply a vacuum and adjust to between -0.1 and -0.2 bar until the methanol has passed through the wells. Open the tap to release the vacuum.
- 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.
- Tap the plate firmly to make sure as much liquid as possible is removed from the bottom of the plate and open the tap to release the vacuum.
- Blot the base of the plate on a paper towel. Discard the waste.

For efficient removal of protein, the membrane should remain wet until the next step.

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.

2 Binding samples

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 glycan samples into each well of the plate, washing out each sample vial with 100 µL of water and adding this to the wells.
- Cover the plate with the lid provided. Leave to bind for 1 hour at room temperature.

If a plate shaker is available, then this can be used to aid binding of the samples. If no plate shaker is available, then the plate can be left on the bench

3 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 and remove the lid. If using the centrifugation method, leave the uncovered protein binding 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 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 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.
- Use a decreased pressure to remove as much water (and drops) from the bottom of plate as possible. Do not tap the plate as this could result in sample contamination. Open the tap to release the vacuum.

4 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. Long drying times at elevated temperatures may lead to glycan desialylation.

Post-endoglycosidase Clean-up procedure

Time Line for Procedure

Procedure	Time
Preparation of PBM plate	5 min
Adding sample	10 min
Eluting glycans	5 min
Drying glycans	as required
Total time	20 min plus drying time

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 dry 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.

Clean-up can be performed by using either any compatible negative pressure system or centrifugation. Centrifugation is recommended for clean-up of samples containing foaming agents such as surfactants. The protocol shown below is for the post-exoglycosidase clean-up using the Ludger vacuum manifold system. **When centrifugation is the preferred method, the vacuum steps can be replaced with 3-minute centrifugation steps at 800 x g speed at room temperature.**

1 Preparation of Protein Binding Plate

Assemble the vacuum manifold with a suitable waste collection container inside. Place the protein binding plate on top of the manifold.

- 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 the methanol has passed through the wells. Open the tap to release the vacuum.
- Pipette 300 µL of water into each well to wash away the methanol. Apply a vacuum and adjust to between -0.05 and -0.2 bar until the water has passed through the wells.
- Tap the plate firmly to make sure as much liquid as possible is removed from the bottom of the plate and open the tap to release the vacuum.
- Blot the base of the plate on a paper towel. Discard the waste.

For efficient removal of protein, the membrane should remain wet until the next step.

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.

2 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 and remove the lid. If using the centrifugation method, leave the uncovered protein binding 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 PBM plate and the collection plate (spacers may be required to lift the collection plate).

- Pipette the released glycan samples into the PBM plate wells. Wash out each sample 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 passed through the wells.Open the tap to release the vacuum.
- Pipette 100 µL of water into each well to wash the membrane and elute any remaining glycans. Apply a vacuum and adjust to between -0.05 and -0.2 bar until the liquid has all gone through the wells.
- Use a decreased pressure to remove as much water (and drops) from the bottom of plate as possible. Do not tap the plate as this could result in sample contamination. Open the tap to release the vacuum.

4 Dry down the samples

At this stage your glycan samples may be sufficiently concentrated for their intended use. If glycans are intended for labelling or other derivatisation, you can transfer the eluted glycan solutions from the collection plate to a PCR plate or sample vials and dry the samples in a vacuum centrifuge. We do not recommend applying heat at this stage. Long drying times at elevated temperatures 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-PBM-96-guide, version v3.0

Appendix 1: Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the PBM kit for glycosidase, protein and peptide removal from underivatised glycans released enzymatically from glycoproteins.

Liquid does not flow.

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

Appendix 2: Material Safety Data Sheet

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Identification of the substance	LudgerClean PBM plates
Composition	Plate of polypropylene containing protein absorption discs.
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.**