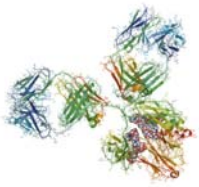

Ludger

Product Guide for Ludger Liberate™ MR Glycan Release Kit

(Ludger Product Guide: LL-MR-A2)

Ludger Document # LL-MR-A2-Guide-v1.2

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Contents

Page

Ludger Liberate MR Glycan Release Kit

Application The Ludger Liberate Membrane Release (MR) kit is used for immobilizing, clean-up and PNGaseF mediated release of glycans from glycoproteins. The ability to immobilize glycoproteins allows the removal of low molecular weight solvent/buffer components, such as trehalose, which may interfere with glycan analysis.

Description The Ludger Liberate MR Glycan Release kit comprises a 96 well membrane-bottom plate and additional reagents for the reduction, alkylation and immobilization of glycoproteins, sample clean up and PNGase F glycan release (PNGaseF supplied separately). **This product is designed for use with a vacuum manifold system, which can be purchased from Ludger but the kit is also compatible with most popular vacuum systems**.

Number of Samples Sufficient for up to 96 samples.

Amount of Sample Up to 100 µg glycoprotein per well, dependent on sample.

Suitable Samples Glycoproteins.

Storage Storage conditions vary. Please check the individual kit components for their storage conditions.

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 and reagents:

Cat. #	Item	Quantity
LL-MR-96	LudgerLiberate 96 Glycan Release Membrane Plate	1
	+ Plate Lid	1
LL-IAA-01	Iodoacetamide 19 mg dried	2
LL-DTT-01	Dithiothreitol 77 mg dried	2
LL-BLOCK-01	Block solution 6 mL liquid	1
LL-FORMIC-01	Formic acid 100 µL liquid	2
LL-NAHCO3-01	20mM sodium bicarbonate 6 mL liquid	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. LL-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).
- PNGase F (E-PNG-05).

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 and any unused chemicals should be discarded according to local safety rules.

Time Line for Procedure

Procedure	Approx Time
1. **Reduction of samples**	20 min
2. **Alkylation of samples**	35 min
3. **Assemble the vacuum manifold**	02 min
4. **Preparation of MR Plate**	05 min
5. **Binding sample**	65 min
6. **Wash the Sample**	10 min
7. **Block the membrane**	65 min
8. **Wash the Sample**	05 min
9. **Incubation with PNGase F to release N-glycans**	10 min plus overnight
10. **Elute N-glycans from MR plate**	10 min
Dry down	04-12 hours
11. **Conversion of released N-glycans to aldoses**	60 min
Dry down	1-4 hours

Method

1 Reduction of samples

Set an oven to 65°C.

Make up 0.5 M DTT by adding 1 mL of water to a vial of 77 mg DTT (**LL-DTT-01**), vortex and briefly centrifuge.

The DTT solution is unstable, it should be used immediately. However, aliquots can be stored frozen for later use.

Add water to make the volume of the glycoprotein up to a total of 200 µl (the sample can be in either a polypropylene tube or a 96 well collection plate).

Add 20 µL 0.5M DTT

Vortex and briefly centrifuge

Incubate at 65C for 15 to 20 min

If a different volume of sample is being used, then add 10% of the samples volume of 0.5M DTT.

2 Alkylation of samples

Make up 100 mM IAA by adding 1 mL water to a vial of 19 mg IAA (**LL-IAA-01**), vortex.

The IAA solution is unstable, it should be used immediately. However, aliquots can be stored frozen for later use.

Remove samples from oven and add 20 µL of the diluted IAA solution.

Vortex and briefly centrifuge

Incubate for 30 min at room temperature in the dark.

If a different volume of sample is being used, then add 10% of the samples volume of 100mM IAA.

The reduced and alkylated sample can be left longer (1-2 hr) at this point if required, or stored overnight at 4°C, or stored frozen at -20°C.

3 Assemble the vacuum manifold

Attach the vacuum manifold to the vacuum pump with the trap kit in between (see the Ludger-Velocity product guide, available on our website, if using the Ludger vacuum manifold system).

Put a collection plate or other suitable container inside the manifold to collect waste.

Put the top back on the manifold.

Place a **LL-MR-96** plate on top of the manifold.

4 Preparation of MR Plate

Pipette 100 µL of methanol into a well for each sample (to wet the membrane).

Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

Pipette 300 µL of water into each well (to wash) Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

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

Use the screw adjuster on the manifold to adjust the pressure to between -3 and -10” Hg (-0.05 and -0.4 bar)*, the maximum pressure used should be no more than 10” Hg (-0.4 bar).*

5 Binding Sample

Remove the plate from the manifold, place on top of a waste collection plate.
Pipette the reduced and alkylated samples into the plate wells.
Cover the plate with the lid.
Place on a shaker for 1 hour at room temperature.

6 Wash the Sample

Put a waste collection plate or other suitable container inside the manifold to collect waste. Place a MR plate on top of the manifold. Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

Pipette 300 µL of water into each well. Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

Repeat with 2 more 300 µL washes of water.

If any of the wells are taking a long time to empty, then opening and closing the vacuum tap to release/apply the vacuum, accompanied by tapping the plate, can help dislodge any blockages.

7 Block the membrane

Shake the blocking solution (**LL-BLOCK-01)** before use.
Pipette 50 µL of blocking solution in to each well.
Place the plate on top of the waste collection plate, cover with the lid.
Place on a shaker for 1 hour at room temperature.

8 Wash the Sample

Put a waste collection plate or other suitable container inside the manifold to collect waste. Place a MR plate on top of the manifold. Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

Pipette 300 µL of water into each well. Apply a vacuum by opening the tap very slowly, and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the liquid has all gone through the well.

9 Incubation with PNGase F to release N-glycans

Defrost **LL-NAHCO3-01** at room temperature.

Calculate the volumes of **E-PNG05 and LL-NAHCO3-01** required for making working solution (0.1U/mL PNGase F) as follows:

a) Total µL of E-PNG05 = number of samples x 1 µL

b) Total µL of LL-NAHCO3-01 = number of samples x 49 µL

Prepare working solution by pipetting **E-PNG05** and **LL-NAHCO3-01** into a tube and mixing.

Remove the MR plate from the manifold and place on top of the clean 96-well 2 mL collection plate (**LP-COLLPLATE-2ML-96**).

Pipette 50 µL of working solution into each well.

Place the lid on the MR plate and seal it with parafilm.

Put the plate on top of the collection plate into a zip lock polythene bag along with a wet tissue and seal.

Incubate at 37°C overnight in a shaking incubator.

The wet tissue in the ziplock bag is to ensure a damp atmosphere to stop the wells drying out. If only a few wells are being used then add 100 µL of water to the surrounding unused wells to help prevent evaporation of samples during incubation.

10 Elute N-glycans from MR plate

Remove the MR plate and 96-well 2 mL collection plate from the incubator.

Place the 2 mL collection plate inside the manifold.

Assemble the manifold with the MR plate on top insuring that the collection plate is in-line with the wells.

Apply a vacuum and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the solution has all gone through the wells.

Pipette 200 µL of water into each well

Apply a vacuum and adjust to between -3 and - 10” Hg (-0.05 and -0.4 bar) until the buffer has all gone through the well.

Repeat with 2 more 200 µL washes of water.

11 Conversion of released N-glycans to aldoses

Dry the eluted glycans in a vacuum centrifuge.

Make up fresh 1% formic acid. Add 10μL formic acid (**LL-FORMIC-01**) to 990μl water in a tube.

Add 20 μL of 1% formic acid to each sample well, vortex thoroughly making sure that the samples are re-dissolved.

Incubate at room temperature for 40 to 50 min on a shaker.

Dry the glycans in a vacuum centrifuge ready for analysis/labelling.

It is important that following the acid incubation do not leave the samples in acid, dry straight away.

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 # LL-MR-A2-guide, version 1.2

Appendix 1: Troubleshooting Guide

The following is a guide to the most likely problems associated with the use of the MR kit for glycan release.

Liquid does not flow.

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

Increase the vacuum pressure if the flow rate is very slow.

Opening and closing the vacuum tap to release/apply the vaccum, accomparied by tapping the plate can help dislodge any blockages

Cross contamination of Samples.

Ensure the wellplate and MR membrane are properly aligned in the manifold.

Make sure there are no drips when moving the MR plate between the waste collection plate and the wellplate.

The samples may spill out of the wells if the speed of the shaker is too high.

Poor Glycan Recovery.

Some glycoproteins can precipitate before immobilization which makes PNGaseF release of the glycans less likely to occur. Reduction and alkylation of the glycoprotein may help overcome this.

In some cases specific glycoproteins (or formulations) may not be amenable for processing using this kit.

Correct ratio of enzyme to buffer is used.

Appendix 2 – Material Safety Data Sheet – LL-MR-96

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	LudgerLiberate MR plates
Composition	Plate of polypropylene containing specialised glycan release 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.**

Material Safety Data Sheet – LL-IAA-01

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	Iodoacetamide, CAS registration number 144-48-9.
Composition	Powder
Hazard indentification	Toxic
First aid measures	ORAL EXPOSURE If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. INHALATION EXPOSURE If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen. DERMAL EXPOSURE In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician. EYE EXPOSURE In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.
Fire fighting measures	Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Specific Hazard(s): Emits toxic fumes under fire conditions. Suitable Extinguising Methods: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.
Accidental release measures	PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL Evacuate area. PROCEDURE(S) OF PERSONAL PRECAUTION(S) Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. METHODS FOR CLEANING UP Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Handling and storage	Store at room temperature. User Exposure: Do not breathe dust. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure. Light sensitive.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Appearance: yellow-brown crystals Melting point: 93 - 95 C
Stability and reactivity	Stable, but light sensitive. Incompatible with strong oxidizing agents, strong bases, reducing agents, acids.
Toxilogical information	Highly toxic. May act as a human carcinogen. This chemical has been shown to induce tumors in laboratory animals when applied to the skin. May cause reproductive damage.
Ecological information	Data not available.
Disposal considerations	Dispose of through controlled chemical disposal handlers. Keep disposal records.
Transport information	Contact Ludger Ltd for transportation information.
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.**

Material Safety Data Sheet – LL-DTT-01

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	Dithiothreitol CAS No: 3483-12-3
Composition	Powder.
Hazard indentification	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	Risk phrases 22-36/37/38
Accidental release measures	Ensure good ventilation.
Handling and storage	Handle with glove and eye protection.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Appearance: white powder with an unpleasant smell Melting point: 41 C
Stability and reactivity	Stable, but heat sensitive.
Toxilogical information	Harmful if swallowed, inhaled or absorbed through the skin. Irritant. Toxicology not fully investigated.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Non-hazardous by air, sea and road freight.
RegulaOther 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.**

Material Safety Data Sheet – LL-BLOCK-01

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	Blocking solution
Composition	Solution.
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	polymer, variable melting point dependent on polymerisation.
Stability and reactivity	Not combustible.
Toxilogical information	Non-harmful-Toxicological, carcinogenic and mutagenic properties have not been investigated.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Non-hazardous.
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.**

Material Safety Data Sheet – LL-NAHCO3-01

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	20 mM sodium carbonate
Composition	Solution.
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	Clear solution**.** Melting point 50oC.
Stability and reactivity	Not combustible.
Toxilogical information	Non-hazardous.
Ecological information	Data not available.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Non-hazardous.
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.**

Material Safety Data Sheet – LL-FORMIC-01

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	Concentrated formic acid
Hazard indentification	Hazardous code C. Corrosive. Large quantities could cause severe burns.
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	Breathing apparatus. Protective clothing.
Accidental release measures	Not applicable.
Handling and storage	Store at room temperature. Handle in a well ventilated area.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Colourless solution. Melting point 8.5oC, boiling point 101oC.
Stability and reactivity	Stable. Substances to be avoided include strong bases, strong oxidizing agents and powdered metals, furfuryl alcohol. Combustible. Hygroscopic.
Toxilogical information	Toxicological, carcinogenic and mutagenic properties have not been investigated.
Disposal considerations	Dispose of through chemical handling waste disposal service.
Transport information	UN No 1779. Hazard class 8.0. Packing group II. Transport category 2.
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.**