Product Guide for LudgerLiberate™ Hydrazinolysis Glycan Release Kit

(Ludger Product Code: LL-HYDRAZ-A2)

Ludger Document # LL-HYDRAZ-A2-Guide-v4.0

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Contents

Specifications for LL-HYDRAZ-A2

Application	For release of N + O-linked glycans from glycoprotein therapeutics
Description	The kit contains reagents for the release of N and O-linked glycans from glycoprotein biopharmaceuticals. Released glycans have free reducing terminii to allow fluorescent tagging by reductive amination. The release conditions can be optimized for release of N-glycans, O-glycans or both N- and O-glycans.
Number of Samples	The kit contains reagents and materials for up to 12 glycoprotein samples analysed in parallel or two sets of 6.
Amount of Sample	Typically, up to 1 mg of glycoprotein per sample.
Suitable Samples	Biopharmaceutical glycoproteins.
Storage:	Store refrigerated at 4 - 10 ^{o}C in the dark. If you have limited cold storage space then store just the CEX cartridges at 4^{o}C and the rest of the kit at room temperature. Protect from sources of heat, light, and moisture. When stored correctly, the reagents should be stable for at least 18 months from date of manufacture.
Shipping:	The product should be shipped at ambient temperature. As it contains small amounts of anhydrous hydrazine the kit must be packaged and shipped as dangerous goods (DG).

For research use only. Not for human or drug use

Kit Contents

The kit contains the following materials and reagents:

Cat. #	Item	Quantity
LL-HYDRAZINE-01	Hydrazinolysis Release Reagent	2 x 3ml
LL-BICARB-01	Solid sodium bicarbonate	2 x 0.33 g
LL-OCTANOL-01	Octanol	2 x 0.2 ml
LL-ACETANHYD-01	Acetic anhydride	2 x 0.5 ml
LL-TFA-5PC-01	TFA solution	2 x 4 ml
LC-EB20-01	LudgerClean™ EB20 cartridges	2 x 6 cartridges
LC-EB20-WASHA-01	EB20 Wash A Solution	2 x 12 ml
LC-EB20-WASHB-01	EB20 Wash B Solution	2 x 12 ml
LC-CEX-H-01	LudgerClean™ CEX cartridges	2 x 6 cartridges
LL-WASTEV-01	Wash waste vials	2 x 6 vials
LL-REACT-01	Glass reaction vials with PTFE lined caps	2 x 6 vials
LL-COLLV-01	Glass collection vials with PTFE lined caps	2 x 6 vials

Additional Reagents and Equipment Required

- Pure water: resistivity 18 MΩ-cm, particle free (>0.22 μm), TOC <10 ppb
- Dialysis membranes, PD10 columns, centrifugal concentrators[§] or similar for removal of salts and detergents from your glycoprotein samples*
- Glove box with dry, oxygen-free inert gas (e.g. nitrogen or argon) for handling anhydrous hydrazine. As an alternative to a glove box, hydrazinolysis can be performed in a dry fume hood with inverted funnel applying a blanket of dry argon.
- Syringe (glass or PTFE) to transfer anhydrous hydrazine – e.g. 1 ml Hamilton or SGE glass syringe for liquids with teflon tipped plunger and stainless steel or PTFE needle. Do not use plastic syringes.
- Heating block, oven, or similar dry heater (a water bath cannot be used) that can be set at between 40^{o}C and 100 ^{o}C
- Centrifugal evaporator (e.g.ThermoSavant SpeedVac®, Heto or GeneVac®). If using the ThermoSavant SpeedVac® we recommend the use of the Thermo Savant RH32-13 Rotor.
- Pipettes

[§] Recommended for glycoprotein sample clean up and buffer exchange prior to O-glycan release

* Optional - depending on your sample

Safety and Handling

- Please read the Material Safety Data Sheets (MSDS's) for all chemicals used (see Appendix 2).

- All processes involving the kit reagents should be performed using appropriate personal safety protection - eyeglasses, good quality chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard.

- Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate.

- All steps involving release reagents must be performed in a dry environment with dry glassware, syringes, needles and plasticware (you can dry these by baking in a warm drying oven). Ensure there are no sources of moisture (e.g. water baths) anywhere near the area used for the hydrazinolysis reaction.

- Once individual vials of reagents are opened, their contents should be used immediately and excess

then discarded according to local safety rules.

The Hydrazinolysis Reaction

The hydrazinolysis reaction involves the following steps (see Figure 1):

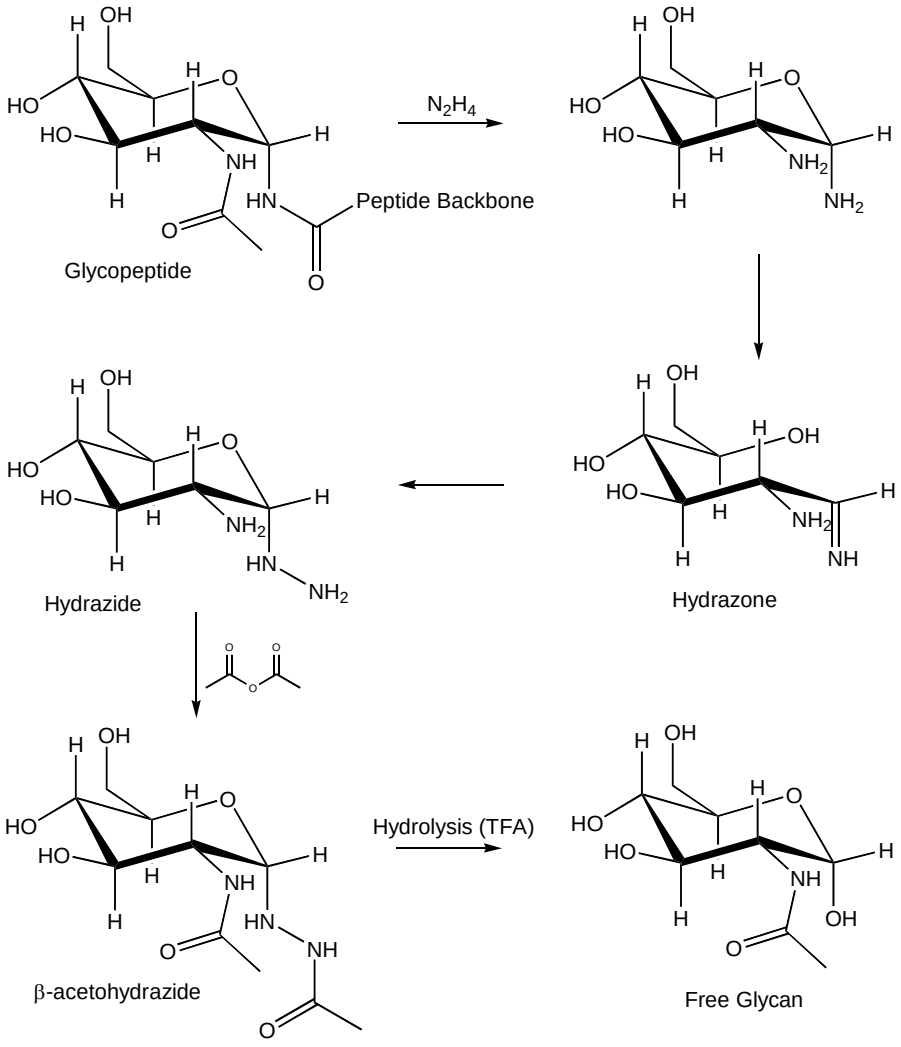


Figure 1: Hydrazinolysis Scheme

This illustrates the scheme for release of an N-linked glycan. The mechanism for hydrazinolyis of O-glycans is similar.

1. **Liberation of the glycan as the hydrazide derivative.**

   Anhydrous hydrazine reacts at (a) the link between the glycan and peptide backbone and (b) the acetamido groups of monosaccharide residues such as GlcNAc. This results in liberation of the glycan as the de-*N*-acetylated hydrazone derivative which then converts to the hydrazide form.

2. ***N*-acetylation.**

   Free amino groups on the hydrazide are *N*-acetylated to form the β-acetohydrazide derivatives. This repairs the de-*N*-acetylated monosaccharide residues and caps the hydrazide moiety of the monosaccharide residue formerly linked to the peptide backbone.

3. **Hydrolysis to form the free glycan.**

   The acid-labile β-acetohydrazide derivative is hydrolysed to produce the free glycan.

Time Line for Hydrazinolysis

Procedure	**Time**
Start with pure glycoprotein samples	
Transfer samples to reaction vials and dry completely	24 hours
Add hydrazine	15 min
Incubate samples *	6 - 16 hour
Remove hydrazine	2-24 hours
Acetylation	2 hours
Purification of released glycans	2 hours

* The incubation time will vary depending on the types of glycans you need to release (i.e. N-glycans, O-glycans or N+O-glycans).

The following are typical incubation regimes:

O-Mode Hydrazinolysis:	Incubate 6 hours at 60^{o}C
N-Mode Hydrazinolysis (Fast):	Incubate 5 hours at 100^{o}C
N-Mode Hydrazinolysis (Normal):	Incubate 16 hours at 85^{o}C
N+O Mode Hydrazinolysis :	Incubate 16 hours at 60^{o}C

The fast and normal N-modes should give equivalent results. Typically, you would choose the fast mode if you plan to work up the samples the same day and the normal mode so the incubation can be done overnight with sample workup the next morning.

Outline of the Hydrazinolysis Protocol

- ## Prepare the glycoprotein

  Prepare the glycoprotein or glycopeptide samples by removing contaminants such as salts, detergents and dyes that could interfere with the release procedure.

- ## Dry the glycoprotein

  Place the samples in reaction vials and dry down completely.

- ## Add hydrazine release reagent

  Add hydrazine release reagent to each sample.

- ## Incubate

  Incubate the sample to allow the release reaction to progress. Use the temperature-time profile optimized for the type of glycans (i.e. N-, O- or N+O) you need to release.

- ## Remove hydrazine

  Remove excess hydrazine by centrifugal evaporation.

- ## Acetylation

  Acetylate free amino groups using acetic anhydride in an aqueous buffer to produce the β-acetohydrazide. Use slightly different protocols depending on the type of released glycans.

- ## Acidification and purification of the free glycans

  Use one of two different protocols for the acidication and purification step, depending on the type of released glycans:

  ### N-Glycans

  Acidify the sample and incubate to produce the free N-glycans

  Purify the N-glycans using an EB20 glycan purification cartridge (Cat # LC-EB20-01)

O-Glycans or N+O-Glycans

Acidify and purify the O-glycans (or N + O-glycans) using a CEX glycan purification cartridge (Cat# LC-CEX-H-01)

- ## Store or analyse released glycans

The released glycans are now ready for direct analysis or fluorescent labelling.

Protocol Steps 1-7 (For All Glycan Types)

Sample Preparation

1 Purify the Glycoprotein

The glycopeptide or glycoprotein samples must be free of contaminants that can interfere with the release reaction. These include the following:

- Non-volatile solvents
- Non-volatile salts, in particular transition metal ions
- Detergents
- Dyes and stains such as Coomassie Blue

Methods that are generally good for removal of such contaminants include the following:

- Dialysis against water or 0.1% trifluoroacetic acid (TFA) as some glycoproteins tend to precipitate in water
- Size exclusion chromatography using a small desalting column (e.g. PD10) with water or 0.1% TFA as eluant
- Centrifugal concentration with buffer exchange into water or 0.1 % TFA

The preferred method for preparing glycoproteins for O-glycan release is centrifugal concentration with buffer exchange into 0.1 % TFA. Washing of the glycoproteins and buffer exchanging them with 0.1 % TFA in this way can significantly decrease the amount of 'peeling product' that is associated with O-glycan release using the hydrazinolysis method.

2 Transfer Samples to Reaction Vials

The amount of sample for each reaction vial (Cat# LL-REACT-01) should be in the range 50 μg to 1 mg.

The reaction vials (5 ml glass vials with Teflon PTFE lined screw caps) included in the kit are pre-cleaned.

3 Dry the Samples

Dry the samples using a centrifugal evaporator or a freeze drier. Samples should be as dry as possible to minimise peeling.

If freeze-drying, be careful to ensure that the sample dries to a small, compact mass at the very bottom of the vial. To do this, freeze dry from a small volume of relatively concentrated glycoprotein solution (typical conditions would be 0.5 mg of glycoprotein sample freeze dried from 200μl of a 2.5 mg/ml solution).
Do not subject samples to high temperatures (> 28 ºC) or extremes of pH as these conditions can result in acid catalyzed loss of sialic acids (high temperatures, low pH) or uncontrolled glycan release (at high pH).

Release Reaction


4 Add Anhydrous Hydrazine

Using a clean, dry glass syringe with PTFE tipped plunger and teflon or stainless steel needle transfer 450 μl of hydrazine reagent (vial LL-HYDRAZINE-01) to each dried sample.
Cap the reaction vials and mix by vortexing.

N.B. This step must be performed in a dry, anaerobic environment - ideally a purpose-built glove box filled with a dry, inert gas (e.g. nitrogen or argon). Alternatively, you can use a dry nitrogen or argon blanket in a chemical fume hood. Contact us for advice.

Ensure that the reaction vial caps are tightly screwed on. For extra security you can seal the caps onto the vials using laboratory PTFE tape. Do not use other laboratory tapes (e.g. Parafilm) that react with hydrazine. Make sure to wind the tape in the same direction as the cap screws onto the vial.

5 Hydrazinolysis Incubation

Place the reaction vials in a heating block, sand tray, or dry oven and incubate according to the type of

glycan release you require:

O-Mode Hydrazinolysis:	Incubate 6 hours at 60°C
N-Mode Hydrazinolysis (Fast):	Incubate 5 hours at 100°C
N-Mode Hydrazinolysis (Normal):	Incubate 16 hours at 85°C
N+O Mode Hydrazinolysis:	Incubate 16 hours at 60°C

The incubation must be performed in a dry environment. Use an oven or dry block - do not use a water bath.

The samples must be completely dissolved in the hydrazine for efficient glycan release. To encourage complete dissolution the samples can be vortexed 15 and 30 minutes after the start of the incubation then the incubation continued.

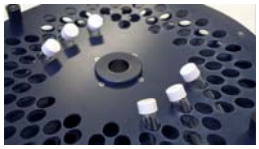
The kinetics of glycan release depends on the type of sample and the glycans. The incubation regimes above give good release for most samples. However, in some cases it may be useful to perform a time-course to optimize the release conditions. When performing a time-course, there are two factors to consider; (a) the yield and (b) side reactions (particularly peeling). The shorter the incubation time, the lower the peeling and lower the yield. Increasing the incubation time increases yield but can result in higher levels of peeling.

The fast and normal N-modes should give equivalent results. Typically, you would choose the fast mode if you plan to work up the samples the same day and the normal mode so the incubation can be done overnight with sample workup the next morning

6 Cool the Samples

After the incubation period, remove the samples from the incubation apparatus and allow them to cool completely to room temperature.

Hydrazine Removal



7 Remove Unreacted Hydrazine

Remove unreacted hydrazine by evaporation in a centrifugal evaporator.

Use gentle heat (maximum 50°C) and a good vacuum. The sample should dry to a small spot at the bottom of the tube.

N.B. Make sure that your centrifugal evaporator is rated to handle hydrazine. Your evaporator should be serviced and clean with good seals. Use an efficient cold trap with temperature -40°C or lower between the evaporation chamber and the pump. Dispose of the cold trap waste according to hazardous waste

regulations. Contact your local waste management service for advice.

Protocol Steps 8-*N* to 16-*N*: (For N-Glycan Release)

Acetylation (N-Glycans)

8-N Prepare Cold 1 M Sodium Bicarbonate Solution

Add 4 ml water to a vial of sodium hydrogen carbonate (bicarbonate - vial LL-BICARB-01) and mix carefully to dissolve all the salt. Cool by placing the vial on ice or in a refrigerator at 0-4 ^{o}C.

9-N Dissolve the Dry Sample in Cold Sodium Bicarbonate Solution

Add 450 μl of cold sodium bicarbonate solution to each dried sample and vortex to mix. Place on ice.

10-N Add Octanol

Add 5 μl of octanol (vial LL-OCTANOL-01) to each vial.

The octanol reduces the foaming and will form a small globule that floats on top of the sample solution

11-N Re-N-acetylation

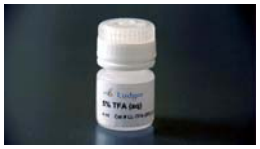
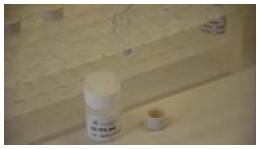
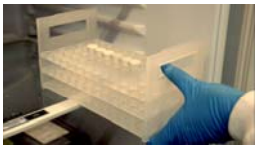
Add 21 μl of acetic anhydride (vial LL-ACETANHYD-01) to each vial, cap and vortex to mix.

You may see small bubbles forming in the sample as the *N*-acetylation occurs. Incubate at 4^{o}C for 1 hour with gentle shaking.

Use a shaker set up that keeps the tubes vertical - e.g. use a test tube rack on a lab 'belly dancer' in a fridge or cold room.

Alternatively, keep the samples in a fridge or on ice and shake gently every 15 minutes.

Acidification (N-Glycans)

12-N Add 5% TFA (aq)

Add 600 µl of 5% trifluoroacetic acid (TFA) solution (vial LL-TFA-5PC-01) to each sample, cap the reaction tube,vortex to mix.

13-N Incubate for Acidification

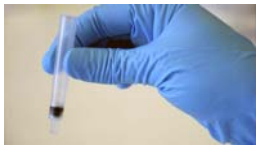
Incubate at 4°C for 1 hour with gentle shaking.

Incubate with shaking as per step 11-N.

This step allows acid catalyzed conversion of the β-acetohydrazones to free unreduced glycans.

The reaction is performed at 4°C to minimize acid catalyzed desialylation.

Post-Release Sample Purification (N-Glycans)





14-N Prime the LudgerClean™ EB20 Cartridges

For each sample, prepare a LudgerClean™ EB20 cartridge (cat # LC-EB20-01) by:

a. placing over a waste vial (cat # LL-WASTEV-01) and

b. washing with 1 x 0.5 ml LC-EB Wash B (vial # LC-EB20-WASHB-01)

c. washing with 1 x 1 ml LC-EB Wash A (vial # LC-EB20-WASHA-01)

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.

Allow each aliquot to flow through the resin bed before the next solution is applied.

15-N Apply the Sample and Wash with Wash A

a. Apply each sample to a primed EB20 cartridge and allow it to flow through the resin bed slowly, under gravity.
b. Wash with 0.7 ml water
c. Wash with 0.7 ml LC-EB20 Wash A (vial # LC-EB20-WASHA-01)

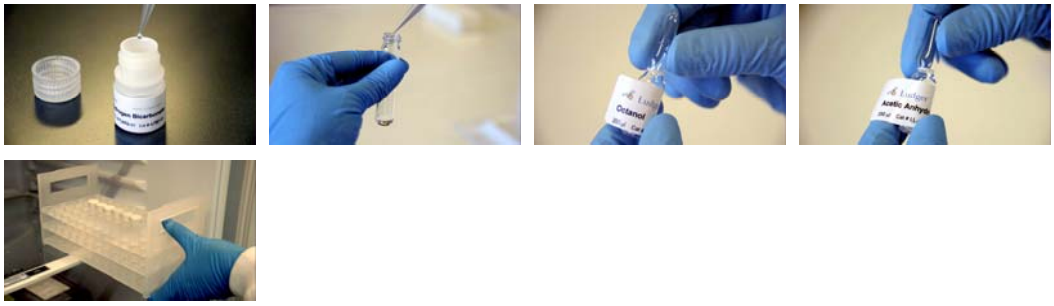
Allow each aliquot to flow through the resin bed before the next solution is applied.
During this step the glycans bind to the resin and salts wash through the cartridge.

16-N Elute Glycans into Collection Vial

a. Place each cartridge over a collection vial (cat # LL-COLLV-01)
b. Elute glycans with 4 x 0.2 ml LC-EB20 Wash B (vial # LC-EB20-WASHB-01) slowly, under gravity.

Protocol Steps 8-*O* to 13-*O:* (For O- and N+O-Glycan Release)

Acetylation (O-Glycans and N+O-Glycans)



8-O Prepare Cold 1 M Sodium Bicarbonate Solution

Add 4 ml water to a vial of sodium hydrogen carbonate (bicarbonate - vial LL-BICARB-01) and mix carefully to dissolve all the salt. Cool by placing the vial on ice or in a refrigerator at 0-4 ^{o}C.

9-O Dissolve the Dry Sample in Cold Sodium Bicarbonate Solution

Add 200 µl of cold sodium bicarbonate solution to each dried sample and vortex to mix. Place on ice.

10-O Add Octanol

Add 5 μl of octanol (vial LL-OCTANOL-01) to each vial.

The octanol reduces the foaming and will form a small globule that floats on top of the sample solution

11-O Re-N-acetylation

Add 21 μl of acetic anhydride (vial LL-ACETANHYD-01) to each vial, cap and vortex to mix. Leave on ice for 10 min, then add a further 21 μl of acetic anhydride (vial LL-ACETANHYD-01) to each vial, cap and vortex to mix. Take off the ice and incubate at room temperature for a further 50 min.

You should see phase-separation between the acetic anhydride and aqueous phases. This helps reduce peeling of O-glycans.

Acidification and Glycan Purification (O-Glycans and N+O-Glycans)


The acidification and post-release sample purification of either O-glycans or N+O-glycans are performed in a single step utilizing the LudgerClean CEX Cartridges

12-O Prime the LudgerClean™ CEX Cartridges

For each sample, prepare a LudgerClean™ CEX cartridge (cat # LC-CEX-H-01) by washing with 10 x 1 ml water

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.
Do not allow the resin to dry out.
Allow each aliquot to flow through the resin bed before the next solution is applied.

13-O Apply the Sample and Elute Glycans

a. Place the cartridges over a collection vial (cat # LL-COLLV-01)
b. Apply each sample to a prepared LudgerClean™ CEX cartridge (cat # LC-CEX-H-01) and allow the solution to flow through the resin bed slowly under gravity.
c. Wash out each vial with 200 µl water and add to the top of each column.
d. Further elute with 3 x 0.5 ml water.

The eluted fluid will contain the purified, released glycans.
If the flow through the column is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.

Proceed to Step 17

Protocol Steps 17 to 18: (For All Glycan Types)

Sample Storage

17 Dry the Glycan Solutions

If required, the samples should be dried by centrifugal evaporation

Keep the sample temperature <28 ^{o}C to minimize desialylation.

This step is optional and can be omitted if you are analyzing aliquots by any method that first involves drying (e.g. addition to a MALDI-MS plate or fluorescence labeling).

Note that at the stage the N-glycans will be in a water-acetonitrile-TFA mixture having been eluted from the EB20 cartridge and O- or N+O-glycans will be in slightly acidic water after elution from the CEX cartridge.

18 Store the Glycans Frozen

For long-term storage, store the glycans at -20^{o}C or lower temperature.

The released glycans can be stored frozen either dried or after reconstitution with water.

Analysis of Released Glycans

The released glycans can be analyzed by a variety of techniques including the following:

- Fluorescence labeling with LudgerTag™ fluorophores followed by HPLC, CE or MS
  The following table lists the current LudgerTag™ fluorophores and rates them according to the suitability for various analysis methods.

Fluorophore	HPLC	MS	CE
2-AB (2-aminobenzamide)	* * * * *	* * *	
2-AA (2-aminobenzoic acid)	* * * * *	* * * * *	* *
AA-Ac (3-(Acetylamino)-6-aminoacridine)	* * * * *	* * * * *	* * * *
APTS (1-aminopyrene-3,6,8-trisulfonate)			* * * * *
2-AP (2-aminopyridine)	* * *	* * * *	

Key:
5 stars = excellent, 4 stars = good, 3 stars = fair, 1 - 2 stars = poor, no stars = not applicable

- Mass spectrometry
- HPAE-PAD (high pH anion exchange chromatography with pulsed amperometric detection)

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-HYDRAZ-A2-Guide-v4.0

Appendix 1: Troubleshooting Guide

The hydrazinolysis protocol is an efficient, robust method. If problems do arise they can normally be corrected without difficulty. The following is a guide to the most likely problems, possible causes, and solutions.

Low Yield

The temperature for hydrazinolysis incubation was incorrect.

Please ensure that the oven or heating block is equilibrated to the incubation temperature and that the reaction tube is subjected to this temperature for the entire release period.

The sample was incompletely solubilized.

The glycoconjugate sample must be completely dissolved in the hydrazine reagent for maximum release efficiency. Please ensure that the sample is thoroughly mixed with the hydrazine reagent prior to the incubation and, as a precaution, re-mix the samples 15 and 30 minutes after the start of the incubation.

The sample contained contaminants that interfered with the release

Ensure that all samples are adequately purified before hydrazinolysis (see protocol step 1).

There was less starting glycoprotein or glycopeptide than was originally estimated.

The glycans were lost during the sample workup

Please ensure that the acidification and glycan purification steps are performed as in the protocol.

Peeling of Glycans

The peeling reaction is degradation of the released glycans characterized by loss of monosaccharide residues from the reducing terminus. O-glycans are generally more susceptible to peeling than N-glycans.

The hydrazinolysis reaction was contaminated with moisture

The incubation of your glycoprotein sample with hydrazine must be done in strictly anhydrous conditions.

- Make sure your sample is completely dry before hydrazinolysis
- Take care to conduct the hydrazine transfer in a dry atmosphere
- Use an absolutely dry transfer syringe.
- Use a dry oven or dry heating block for the incubation.

The hydrazinolysis mode did not match the type of glycans

O-glycans will peel, degrade and be lost under N-mode hydrazinolysis conditions.

N-glycans will not be removed efficiently under O-mode hydrazinolysis conditions

The hydrazinolysis temperature-time profile was too harsh for the glycans

Use the temperature-time profiles given in this protocol as a starting point. If you see peeling that is not related to moisture contamination then for subsequent experiments reduce the temperature or time for the hydrazine incubation.

Desialylation of the Glycans

The sample was subjected to acidic conditions in aqueous solutions at elevated temperatures

Avoid prolonged periods of exposure of sialylated glycan or glycoprotein samples in aqueous solutions at low pH and elevated temperatures.

In general, try to keep samples in solutions in the pH range 5 – 8.5 and avoid exposure to temperatures above 28 ^{o}C. Samples in pH buffered aqueous solutions (with pH between 5 and 8.5) tend to be resistant to acid catalyzed de-sialylation even at temperatures higher than 28 ^{o}C. However, even then it is wise to err on the side of caution and keep the samples cool whenever possible.

Contamination with Non-Sample Related Sugars

The sample has been contaminated with environmental sugars

Ensure that all possible sources of sugar contamination are eliminated or contained.

Common sources of contamination include the following:

- Powdered laboratory gloves – the powder often contains starch so use powder-free gloves in glycan analysis labs
- Sugars from food contamination on clothing, hair and skin. Avoid handling sugary foods (e.g. doughnuts) and wash your hands thoroughly before performing glycan analyses (glycoprofiling can help promote a clean and healthy way of living).

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Cannot Assign Peaks on Samples Analyzed by HPLC, MS or CE

Use glycoprotein and glycan standards appropriate for your project

Select reference glycoprotein standards to use as positive controls for hydrazinolysis and use relevant glycan standards in subsequent analyses. Ludger is developing a range of matched glycoprotein and released glycans as certified reference standards for use in glycoprofiling studies. Please contact us for advice on what standards to use for your particular application.

Appendix 2: Material Safety Data Sheets

The advice offered in the following material safety data sheets (MSDS's) 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.

The following notes apply to all materials listed in the following MSDS's:

Transport information

Contact Ludger for transportation information.

Abbreviations

GLP	Good Laboratory Practice

The advice offered in the following material safety data sheets (MSDS's) 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.

The following notes apply to all materials listed in the following MSDS's:

Transport information

Contact Ludger for transportation information.

Abbreviations

GLP	Good Laboratory Practice

Material Safety Data Sheet: LL-HYDRAZINE-01

Manufacturer Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK
Tel: +44 870 085 7011 Fax: +44 870 163 4620 www.ludger.com

Identification of the substance and Composition

Anhydrous hydrazine (NH_2NH_2) Chemical name: Hydrazine. CAS no. 302-01-02

Hazard identification

Strong reducing agent. Fire and explosion risk in contact with oxidizing agents. May be fatal if absorbed through the skin. Causes eye and skin burns. Causes digestive and respiratory tract burns. Flammable liquid and vapor. Harmful if inhaled or swallowed. May cause allergic skin reaction. Cancer suspect agent. May cause blood abnormalities. May cause liver and kidney damage. *Target Organs*: Blood, kidneys, liver.

First aid measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid immediately. Do NOT allow victim to rub eyes or keep eyes closed.

Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Discard contaminated clothing in a manner which limits further exposure.

Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If breathing is difficult, give oxygen. Do NOT use mouth-to-mouth resuscitation. If breathing has ceased apply artificial respiration using oxygen and a suitable mechanical device such as a bag and a mask.

Notes to Physician: Treat symptomatically and supportively.

Fire fighting measures

Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures

Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage

Keep away from heat, sparks, and flame. Keep away from sources of ignition. Do not store in direct sunlight. Store in a cool, dry area away from incompatible substances. Isolate from oxidizing materials and acids. Handle in accordance with GLP.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties

Colourless liquid. Strong odor - ammonia-like.

Stability and reactivity

Thermally unstable. Avoid contact with oxidizing agents, ignition sources, temperatures above 150^{o}C,

moisture.

Toxicological information

Harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.

Ecological information

Data not available.

Disposal considerations

Dilute with excess water, mop up with absorptive material and dispose of as hazardous material according to local regulations.

Regulatory

Risk Phrases:

R 10 Flammable.

R 23/24/25 Toxic by inhalation, in contact with skin and if swallowed.

R 34 Causes burns.

R 43 May cause sensitization by skin contact.

R 45 May cause cancer.

R 50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety Phrases:

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S 53 Avoid exposure - obtain special instructions before use.

S 60 This material and its container must be disposed of as hazardous waste.

S 61 Avoid release to the environment.

Material Safety Data Sheet: LL-BICARB-01

Manufacturer	Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com
Identification of the substance	Sodium hydrogen carbonate; sodium bicarbonate
Composition	$NaHCO_3$ Chemical name: sodium hydrogen carbonate. CAS no. 144-55-8

Hazard identification

Causes eye irritation. May cause skin and respiratory tract irritation.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

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

Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures

Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage

Store in a cool place or at room temperature. Handle in accordance with GLP.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety specs, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties

White powder

Stability and reactivity

Stable in dry air, but slowly decomposes in moist air

Toxicological information

May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.

Disposal considerations

Dilute with excess water, mop up with absorptive material and dispose of according to local regulations.

Regulatory information

Risk Phrases: *None*

Safety phrases

S 24/25 Avoid contact with skin and eyes.

Material Safety Data Sheet: LL-OCTANOL-01

Manufacturer Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK
Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com

Identification of the substance 1-Octanol

Composition $C_8H_{18}O$ Chemical name: Octanol CAS no. 111-87-5

Hazard identification Irritant.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

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

Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures

Wear appropriate protective clothing. As quantities are small absorb sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage

Store at room temperature. Handle in accordance with GLP.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties

Clear, colourless liquid. Penetrating, sweetish odor. Combustible liquid and vapor. Flash point: 81^{o}C

Stability and reactivity

Stable under normal temperatures and pressures. Avoid ignition sources, excess heat, exposure to strong oxidizing agents

Toxicological information

May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.

Ecological information Data not available.

Disposal considerations

Dilute with excess water, mop up with absorptive material and dispose of according to local regulations.

Transport information Contact Ludger for transportation information.

Regulatory information

Risk phrases :

R 36/38 Irritating to eyes and skin.

Safety phrases :

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 37/39 Wear suitable gloves and eye/face protection.

Material Safety Data Sheet: LL-ACETANHYD-01

Manufacturer	Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com
Identification of the substance	Acetic anhydride
Composition	Chemical name: Acetic anhydride CAS no. 108-24-7
Hazard identification	Corrosive. Irritant. Flammable liquid and vapor (flash point 52 ^{o}C)

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures

Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures

Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage: Store at room temperature. Handle in accordance with Good Laboratory Practice.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties	Colourless liquid. Stong pungent acetic odor.
Stability and reactivity	Stable. Readily hydrolyzed in water to form corresponding acid.

Toxicological information

May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.

Disposal considerations: Dilute with excess water and dispose of according to local regulations.

Transport information	Contact Ludger for transportation information.

Regulatory information

Risk phrases :

R 10 Flammable.

R 20/22 Harmful by inhalation and if swallowed.

R 34 Causes burns.

Safety phrases :

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S 45 In case of accident or if you feel unwell, seek medical advice immediately.

Material Safety Data Sheet: LL-TFA-5PC-01

Manufacturer	Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com
Identification of the substance	5% Trifluoroacetic acid (aq)
Composition	Trifluoroacetic acid (5% v/v), water (95% v/v) Chemical name: Trifluoroacetic acid. CAS no. 76-05-01
Hazard identification	Both liquid and vapor can cause burns to all parts of the body.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures

Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures	Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties	Colourless liquid.
Stability and reactivity	Stable under normal temperatures and pressures.
Toxicological information	May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.
Disposal considerations	Dilute with excess water, mop up with absorptive material and dispose of according to local regulations.
Transport information	Contact Ludger for transportation information.

Regulatory information

Risk phrases :

R 35 Causes severe burns.

R 20 Harmful by inhalation.

R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases :

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 27 Take off immediately all contaminated clothing.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S 9 Keep container in a well-ventilated place.

S 28A After contact with skin, wash immediately with plenty of water

Material Safety Data Sheet: LC-EB20-WASHA-01

Manufacturer Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK
Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com

Identification of the substance LudgerClean EB20 Wash A

Composition

5% acetonitrile, 0.1% trifluoroacetic acid

Chemical name: Acetonitrile (CAS no. 75-05-08), Trifluoroacetic acid. (CAS no. 76-05-01)

Hazard identification Irritant.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures: Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures

Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage : Store at room temperature. Handle in accordance with Good Laboratory Practice.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties Colourless liquid.

Stability and reactivity Stable under normal temperatures and pressures.

Toxicological information

May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation,

Disposal considerations

Dilute with excess water, mop up with absorptive material and dispose of as hazardous solvent waste according to local regulations.

Transport information Contact Ludger for transportation information.

Regulatory information

Risk phrases :

R 11 Highly flammable.

R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

R 36 Irritating to eyes

Safety phrases :

S 16 Keep away from sources of ignition - No smoking.

S 36/37 Wear suitable protective clothing and gloves.

Material Safety Data Sheet : LC-EB20-WASHB-01

Manufacturer Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK
Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com

Identification of the substance 50% acetonitrile, 0.1% trifluoroacetic acid (aq)

Composition

Chemical names: Acetonitrile. (CAS no. 75-05-08), Trifluoroacetic acid (CAS no. 76-05-01)

Hazard identification Irritant.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures: Water spray or appropriate foam according to surrounding fire conditions.

Accidental release measures Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.

Handling and storage : Store at room temperature. Handle in accordance with Good Laboratory Practice.

Exposure Controls / Personal Protection

Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.

Physical and chemical properties Colourless liquid.

Stability and reactivity Stable under normal temperatures and pressures.

Toxicological information

May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation,

Disposal considerations

Dilute with excess water, mop up with absorptive material and dispose of as hazardous solvent waste according to local regulations.

Transport information Contact Ludger for transportation information.

Regulatory information

Risk phrases :

R 11 Highly flammable.

R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

R 36 Irritating to eyes

Safety phrases :

S 16 Keep away from sources of ignition - No smoking.

S 36/37 Wear suitable protective clothing and gloves.