PROzyme®

GlycoRelease™

GLYCAN HYDRAZINOLYSIS KIT

Rapid, reliable and essentially quantitative non-selective release and recovery of intact glycans from glycoproteins using hydrazinolysis.

- Cleavage independent of the glycan structure or the primary sequence of the glycoprotein
- Glycan recovery typically >90%
- Released glycans contain a free reducing end
- Cleavage differentiation between N- and O-linked glycans
- Sample size: 50 - 500 µg
- Up to 18 individual samples (3 batches of 6)
- Includes a license to use

Product Code GK50202

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

Agilent

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This product is intended for *in vitro* research use only.

KIT CONTENTS

NOTE: Please read this entire booklet before starting your experiments to obtain successful results.

NOTE: Hydrazine is shipped in a separate package. Make sure all components are received before proceeding.

Item	Qty
H1 Cleavage Reagent (anhydrous hydrazine, supplied in ampules)	3 ea
H2 Re-N-acetylation Buffer	1 ea
H3 Re-N-acetylation Reagent (supplied in ampules)	3 ea
H4 Desalting Resin	1 ea
Reaction Vials (set of 6, with screw caps)	3 ea
Spin Filter Cartridges (set of 6, with collection tubes)	3 ea
Spin Filter Cartridges (set of 3, with collection tubes)	1 ea
Bovine Fetuin Control	1 ea

Storage Conditions

Store at room temperature upon receipt.
Store bovine fetuin, lyophilized or in solution, at -20°C.

Additional Required Reagents/Equipment

Glass syringe, gas-tight with Teflon® plunger (*i.e.* Hamilton #81101)
Lyophilizer
Desiccator with suitable drying agent
Heating block (for 12-mm vials), sand bath or dry oven set to 60°C or 95°C
CAP Type I water supply (Milli-Q or equivalent)
Dialysis membrane (5 - 10 kDa cutoff)
SpeedVac® evaporator or equivalent with a rotor for 12-mm (outer diameter) vials or vacuum evaporator with charcoal/alumina trap.
Vortex mixer
Dry nitrogen or argon (optional)

SAFETY AND HANDLING

Please read the Material Safety Data Sheets (MSDS) included in the packages. All procedures involving kit reagents should be performed using appropriate personal safety protection, including lab coat, eyeglasses, chemically resistant (*e.g.* nitrile) gloves and, where appropriate, in a laboratory fume hood.

H1 Cleavage Reagent (hydrazine) is corrosive, toxic and flammable, and should be handled in a fume hood while wearing eye protection and gloves. A fresh ampule must be used for each set of deglycosylation reactions; the reagent must be discarded after it has been exposed to the atmospheric moisture for longer than 2 minutes. For disposal, see the Material Safety Data Sheet.

General Laboratory Procedures

Use powder-free gloves for all sample handling procedures. Make sure that any glassware, plasticware, solvents or reagents used in addition to the kit components are free of glycosidases and carbohydrate contaminants.

All steps involving hydrazine must be performed in a dry environment with dry glassware; moisture will cause degradation of glycans (especially O-linked glycans) during the hydrazinolysis reaction. Glassware might be either pyrolyzed (500°C) or acid washed (sonicated for 10 minutes in 2 *M* nitric acid), then dried thoroughly, preferably in a vacuum oven.

Once individual hydrazine ampules are opened, their contents should be used immediately and the excess discarded.

LICENSE TO USE

By accepting delivery of the GlycoRelease Glycan Hydrazinolysis Kit [Material(s)] and by subsequently using the Material(s) to release glycans, Recipient agrees to be bound by the following terms and restrictions:

1. A Use Sublicense is granted Recipient for in-house use of Material(s) only. This Use Sublicense is defined in the intellectual property license agreement between Biomarin Pharmaceutical and ProZyme.

2. The Material(s) will not be made available by Recipient to any third parties in any form, separately or in combination, for any monetary or other consideration or at no charge, except that the Material(s) may be made available to third parties who agree to be bound by all the terms and restrictions of this Agreement for purposes of evaluation only.

3. The Material(s) and the released glycans will not be used *in vivo* in humans.

4. Recipient will not make commercial use of the Material(s) unless it first secures a license agreement from ProZyme, Inc. for such commercial use.

INTRODUCTION

Treatment of glycoproteins, glycolipids, and other glycoconjugates with anhydrous hydrazine under controlled conditions, has proven to be an effective and valuable method for the non-selective, yet uniform release of intact, free reducing end-containing N- and/or O-linked glycans, in high yield (Patel and Parekh, 1994 and Patel *et al*., 1993). It is a particularly powerful component of glycoconjugate composition and structural analytical strategies (Royle *et al*., 2002). Since the glycans released by hydrazinolysis contain a free reducing end, they can readily be derivatized with colorimetric or fluorescent tags, allowing their precise qualitative, quantitative and structural analysis when used in conjunction with a variety of chromatographic (Anumula and Dhume, 1998; Townsend *et al*., 1996; Bigge *et al*., 1995 and Guile *et al*., 1996) and/or mass spectrometry techniques (Royle *et al*., 2002). In contrast, other traditional methods of cleavage of O-linked glycans, such as alkali-based reductive β-elimination, produce only reduced glycans, which cannot be easily derivatized.

Hydrazinolysis using the standard reaction conditions has been extensively compared to enzymatic deglycosylation, and found to be generally applicable to a wide range of glycoproteins. The hydrazine molecule is small and release of glycans by hydrazinolysis does not generally seem to be significantly influenced by steric factors. However, a small decrease in the yield of O-glycans due to clustering of O-glycosylation sites has been reported for hydrazinolysis of human glycophorin A (Merry *et al*., 2002).

At elevated temperature and extended reaction time, hydrazinolysis is non-selective towards either the glycan structure or the primary sequence of the protein, cleaving both O- and N-linked structures with efficiencies approaching 90% or greater. However, with careful control of reaction conditions, selectivity of release of either O- and/or N-linked glycans can be achieved.

Mechanism of Action

The precise mechanism by which hydrazine cleaves all types of glycans from glycoproteins is not completely understood; a proposed reaction scheme for the release of N-linked oligosaccharides from glycoproteins is shown in Figure 1. According to this model, hydrazine cleaves the glycosylamine linkage and releases an intact glycan from the asparagine attachment site on the polypeptide chain (Step 1).

The terminal N-acetylglucosamine residue of the released glycan forms a transient covalent adduct with hydrazine (β-acetohydrazide). Concomitantly, as a side reaction, the N-acetyl moieties of all amino sugars are hydrolyzed under these conditions. Thus, after hydrazinolysis, re-acetylation of the free amino groups with acetic anhydride or other suitable acetylation reagent is necessary to convert the amino sugars back to their original acetylated state (Figure 1, Step 2).

Re-N-acetylation also results in a cleavage of the β-aceto-hydrazide derivatives. However, a small amount of these adducts may remain after the re-N-acetylation reaction. The complete regeneration of the reducing end of the glycans can

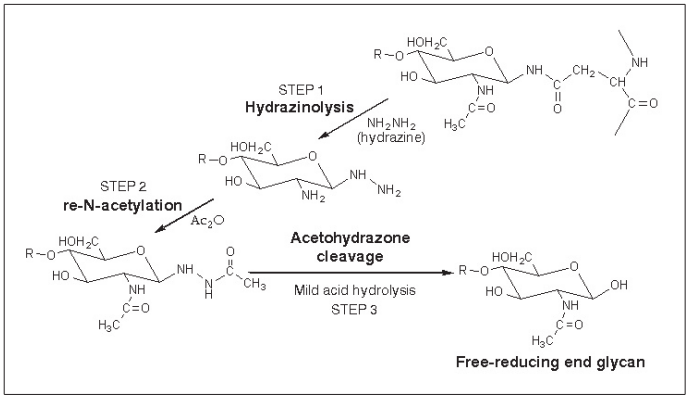

Figure 1 - Proposed reaction for the release of N-linked glycans by hydrazine.

be achieved by mild hydrolysis with mineral or Lewis acid (such as copper acetate) as shown (Figure 1, Step 3).

A possible reaction mechanism for the cleavage of O-linked glycans from glycoproteins includes hydrazine-catalyzed β-elimination of the glycan and formation of hydrazone derivatives through the reaction of released glycans with the excess of hydrazine. An undesirable side reaction can occur due to β-elimination of the monosaccharide at the reducing end of the released O-glycan chains, commonly referred to as degradative peeling.

Recent studies have found that the degree of peeling of O-linked glycans can be greatly minimized by maintaining anhydrous conditions, selection of the appropriate buffer for re-N-acetylation, and careful temperature control (Merry *et al.*, 2002).

Overall, hydrazinolysis is relatively moderate in its action and sensitive carbohydrate residues, such as sialic acid, are not degraded. However, hydrolysis of certain N- and O-acyl substitutions in the sialic acids can be anticipated since they are known to be relatively labile under basic conditions.

Finally, substantial cleavage of peptide bonds occurs during hydrazinolysis, therefore hydrazinolysis should not be used when recovery of the intact protein portion is desired.

Conditions of Hydrazinolysis

Reaction conditions are listed in Table 1 and the choice depends on the type of cleavage desired. O-linked glycans are released during hydrazinolysis at a lower temperature than N-linked glycans and, once released, are susceptible to degradation at the higher temperature required for the cleavage of N-linked glycans. The choice of glycan cleavage approaches includes:

- selective hydrazinolysis at 60°C for 6 hours, during which ≥90 of O-linked glycans are released, but <10% of N-glycosidic bonds are cleaved (if present on the starting glycoprotein).

- hydrazinolysis at 95°C for 5 hours, during which ≥85% of both O- and N-linked glycans are released.

- sequential hydrazinolysis of O-linked and then N-linked glycans from the same aliquot of glycoprotein.

Another potential strategy for sequential release of O–linked and N-linked glycans from glycoproteins comprises an enzymatic release of N-linked glycans with N-Glycanase® followed by hydrazinolysis of remaining O-linked glycans.

Analysis of Released Glycans

After isolation and purification, glycans released from the starting glycoprotein can be analyzed in a number of ways:

- derivatization with a fluorescent dye, such as ANTS (8-aminonaphthalene-1,3,6-trisulfonic acid), and the individual glycan structures electrophoretically resolved, quantified or sequenced using FACE® (Fluorophore-assisted Carbohydrate Electrophoresis, available from ProZyme).

- derivatization with fluorescent dyes, such as 2-AB (2-aminobenzamide, Signal™ 2-AB Labeling Kit, product code GKK-404, available from ProZyme), and the individual glycan structures chromatographically resolved, quantified or sequenced using GlycoSep™ HPLC columns (available from ProZyme).

In addition, mass spectrometry can greatly assist in the determination of composition and structure of the labeled glycans.

Table 1 - Hydrazinolysis Reaction Conditions

Glycan Release	Temperature	Incubation Time
O-Linked	60°C	6 hours[1] (4 - 6 hours)[2]
N-Linked	95°C	5 hours (short)[3] 12 - 16 hours (long)[4]
N- & O-Linked	95°C	4 hours[5]

[1] (recommended) see Merry *et al.*, 2002

[2] 4 - 6 hours, used in various studies. 5 hours, ≥90% O-linked glycans released and ≤10% N-linked glycans released.

[3] these conditions should result in ≥90% N-linked glycans released, with partial degradation of O-linked glycans.

[4] long incubation will result in a lower recovery of O-linked glycans.

[5] these conditions constitute a compromise and should result in ≥85% of O- & N-linked glycans released.

USING THE KIT

The H1 Glycan Cleavage Reagent (Hydrazine) ampules are shipped in a separate package; they are surrounded by vermiculite and sealed in a bottle within an aluminum can. Take care when unpacking as the ampules are fragile.

Up to 6 samples may be subjected to hydrazinolysis using one set of reagents; a fresh H1 Cleavage Reagent ampule must be used for each batch of samples. H1 should be discarded after exposure to atmospheric moisture for longer than 2 minutes. Three ampules of H1 have been included to allow more flexibility for the user, as well as to minimize unnecessary disposal of hydrazine, which constitutes an environmental hazard.

Opening the Component Ampules

Make sure to take proper safety measures for handling the contents as described in the enclosed MSDS's. *Be sure to wear gloves and safety glasses during these operations*. Gently tap the ampule to settle the contents on the bottom. To open, hold both the body and the top of the ampule, then gently but firmly snap open at the break-ring. Snap away from your body. Beware of the sharp edges of the opening.

Reaction Vials

While supplied clean, the user may wish to remove any traces of potential carbohydrate contamination by pyrolysis (500°C) or acid wash (sonication for 10 minutes in 2 *M* nitric acid), then thorough drying, preferably in a vacuum oven.

The Reaction Vials are 12 mm wide. Make sure the heating block for incubation of the reactions and Speed-Vac rotor for centrifugal evaporation of the final product samples have holes wide enough to accommodate the vials.

Using the Syringe

The H1 Glycan Cleavage Reagent (Hydrazine) should be dispensed using a chemically inert, gas-tight, glass syringe, fitted with a Teflon plunger tip and stainless steel needle that has been thoroughly dried.

Dry the syringe thoroughly prior to use: wash with reagent-grade acetone, flush with a stream of dry nitrogen, warm in a dry oven (~90°C), cool and store in a desiccator. The use of a glass, Luer-tipped syringe with a removable needle is recommended since both can be oven-dried at 90°C independently, without damage to the syringe. Please note that a significant dead-volume in the needle occurs with this arrangement; remove all air from the needle before transferring a solution by aspirating the liquid up and down into the syringe body several times.

NOTE: Disposable plastic syringes with a rubber-tipped plunger are not recommended as they usually contain lubricant, which may contaminate the reaction mixture or be degraded when placed in contact with hydrazine.

Using the Bovine Fetuin Control

Dissolve the fetuin control in 200 µl of ultrapure water. Use 20 - 100 µl (100 - 500 µg) per analysis. Store any unused remainder at -20°C.

PROTOCOL

Outline of the Procedure

1. Sample Preparation

   Glycoproteins or glycopeptides (free of salts, metal ions and detergents) are thoroughly dried in the Reaction Vials provided.

2. Cleavage of Glycans

   Samples are incubated with hydrazine; the type of glycans released depends on the temperature and incubation time selected (Table 1).

3. Re-N-acetylation of Released Glycans

   Glycans, which were de-acetylated during hydrazinolysis, are re-N-acetylated. This step will also result in acetylation of the amino groups of the peptides/protein.

4. Recovery of Released Glycans

   Glycans are desalted, filtered and dried.

Samples consist of re-N-acetylated glycans containing free reducing ends and deglycosylated peptides.

Sample Preparation

The preparation of the sample is crucial to a successful outcome. Samples should be rendered as free of salts as is practical and thoroughly dried before hydrazine addition. The preferred approach for removing salts and some detergents is dialysis at 4°C with an appropriate molecular weight cutoff (5 - 10 kDA) membrane against water; dilute acid (0.1% [v/v] trifluoroacetic acid or 1% [v/v] acetic acid); or volatile buffer (low (<0.1*M*) molarity ammonium bicarbonate or ammonium acetate).

NOTE: Dilute acids do not cause any detectable loss of acid-labile carbohydrate determinants, such as sialic acids, if dialysis is performed at 4° C and samples are lyophilized immediately following dialysis.

NOTE: Dialysis buffer should be chosen to maintain the sample in solution, although this is not essential as shown by successful hydrazinolysis on crude or relatively insoluble samples.

NOTE: Carbohydrate-based resins should be avoided for glycoprotein purification as matrix breakdown products cannot be removed by dialysis.

NOTE: Most detergents can be removed from glycoprotein samples by reverse-phase HPLC. Deoxycholate interferes with hydrazinolysis, but low levels (~1%) of SDS or Tween® 80 can be tolerated.

Reagents and Supplies

Samples - solutions of glycoproteins or glycopeptides free of salts, metal ions and/or detergents (up to six for each set of reagents). Sample quantities should be in the range of 50 - 500 µg, depending on the extent of glycosylation.

NOTE: the smallest amount of sample is largely dependent on the minimum amount of glycan that can be analyzed by the user with the techniques at their disposal. The loss of glycans during the protocol is relatively small.

Reaction Vials (supplied with the kit)

Procedure

Transfer each sample solution (≤100 µl) to a Reaction Vial, cap loosely and lyophilize thoroughly.

NOTE: Samples must be thoroughly dry prior to hydrazine addition to prevent glycan degradation. To ensure that the drying step reaches completion, extended lyophilization of samples is recommended (≤ 50 milliTorr; ≥ 24 hours).

If excessive glycan degradation is observed, lyophilized samples should be further dried overnight under high vacuum in the presence of fresh phosphorous pentoxide (P_2O_5).

Remove the sample from the lyophilizer/desiccator, cap the Reaction Vial tightly and proceed immediately to hydrazinolysis. Do not store for any period of time as significant water uptake may occur.

Hydrazinolysis Reaction

CAUTION: Do not expose the hydrazine to any source of ignition. Do not expose the sample or the hydrazine to moisture.

Reagents and Supplies

Dry samples in Reaction Vials (from Sample Preparation)

H1 Cleavage Reagent (one ampule for each set of 6 samples, supplied with the kit)

Heating block or sand bath. *Do Not Use a Water Bath!*

Gas-tight, glass syringe with Teflon-tipped plunger and stainless steel needle (clean and thoroughly dried) stored in a desiccator.

Procedure

Choose the Hydrazinolysis Reaction Conditions from Table 1; set the heating block to the selected temperature.

Remove the syringe from the desiccator just prior to opening an ampule of H1 Cleavage Reagent.

Tap the H1 ampule gently to ensure that the contents are at the bottom. Break open the ampule.

Add 60 µl of H1 to each sample using the (dry) syringe and tightly cap each Reaction Vial.

Optional: Overlay the sample with dry nitrogen or argon and cap tightly.

NOTE: Hydrazine is very hygroscopic. Use within 2 minutes and discard any unused hydrazine according to your hazardous waste regulations. DO NOT REUSE.

Gently mix the contents of each vial (vortex). Shake down each vial to ensure that the sample/hydrazine remains at the bottom of the vial and not on the sides or cap.

Incubate the samples for the selected Incubation Time.

Allow the Reaction Vial to cool. Remove hydrazine by evaporation using a SpeedVac (~30 minutes or until dry) or a high-vacuum pump fitted with an activated charcoal/alumina trap.

NOTE: If using a SpeedVac, turn heat setting to off position.

Re-N-acetylation Reaction

Reagents and Supplies

H2 Re-N-acetylation Buffer (supplied with the kit)

H3 Re-N-acetylation Reagent (supplied with the kit)

H4 Desalting Resin (supplied with the kit)

Spin Filter Cartridges - 6 pack (3 ea, supplied with the kit)

Spin Filter Cartridges - 3 pack (supplied with the kit)

Microcentrifuge

Vortex mixer

Preparation of H4 Desalting Resin

NOTE: Due to the strong anionic character of the H4 Desalting Resin, partial leaching can be observed over time. Prepare the resin just prior to use to avoid desialylation and other potential glycan degradation.

Add 800 µl of water to the tube of H4 Desalting Resin (total volume of resin suspension will be ~1.5 ml). Mix by pipetting up and down several times.

Transfer 500 µl of H4 Resin suspension to each of the three Spin Filter Cartridges (3 pack).

Centrifuge at 10,000 rpm in a microcentrifuge for 2 minutes. Discard the flow-through.

NOTE: Each of the three aliquots of H4 Resin is intended to be used for one set of 6 reactions. Unused aliquots should be stored drained in the Spin Filter Cartridge at 4° C and washed just prior to use.

To wash the resin, add 500 µl of water to the spin filter cartridge and vortex briefly.

Centrifuge at 10,000 rpm in a microcentrifuge for 2 minutes. Discard the flow-through. Repeat four times.

Re-N-acetylation Procedure

Add 40 µl of H2 Re-N-acetylation Buffer to each Reaction Vial. Cap the vial and mix gently to resuspend the sample.

Tap H3 Re-N-acetylation Reagent gently to ensure that the contents are at the bottom. Break open the ampule.

Add 10 µl of H3 to each Reaction Vial. Cap the vial and mix strongly (vortex) for 1 minute.

NOTE: Vials should be capped during strong mixing. Mixing of the vial without the cap may result in separation of the fused glass insert.

Incubate for 1 hour at room temperature (repeat mixing after initial 15 minutes of incubation).

Add 270 µl of water to the spin filter cartridge containing the washed Resin. Mix by pipetting up and down several times.

NOTE: Resin settles rapidly. Mix suspended resin in a circular motion with the pipette tip while withdrawing from the bottom of the cartridge. To dispense reproducible aliquots, resuspend the resin in the cartridge by mixing after each pipetting.

Withdraw 60 µl of the H4 Suspension and add to each Reaction Vial.

Cap each vial and mix strongly for 1 minute (vortex).

Transfer the entire contents from each Reaction Vial (including the suspended resin) to a clean spin filter cartridge.

NOTE: Before transfer, pipette up and down several times to resuspend the resin.

Wash each Reaction Vial twice with 50 µl of water (to maximize sample recovery) and add both washes to the sample/resin suspension in the spin filter cartridge.

Centrifuge at 10,000 rpm in a microcentrifuge for 3 minutes or until the resin has been completely drained. (DO NOT DISCARD the flow-through in the collection tube: these are the released glycans!)

Add 150 µl of water to the resin in each spin filter cartridge and vortex gently to mix the resin. Centrifuge at 10,000 rpm in a microcentrifuge for 3 minutes or until the resin has been completely drained. (DO NOT REMOVE the flow-through from the collection tube.)

Add an additional 150 µl of water to the resin in each spin filter cartridge. Repeat centrifugation (previous step).

Keep each collection tube and discard the spin filter cartridges. Dry the combined flow-through using a SpeedVac evaporator (without heating).

NOTE: Samples at this stage consist of re-N-acetylated glycans containing a free reducing end and deglycosylated peptide/protein.

Recovery of Oligosaccharides

If analyzing the glycans using FACE, proceed directly to labeling; the presence of peptides does not interfere with FACE.

If desired, released glycans can be separated from peptide-derived material using paper chromatography (Royle *et al*., 2002) or glass bead chromatography (Merry *et al*., 2002).

EXAMPLE: RELEASE OF O-LINKED GLYCANS FROM FETUIN

Bovine fetuin has three N- and three O-glycosylation sites, and can be used as a control to evaluate the quality of the release of O-linked glycans. In this example, O-glycans were released from 100 µg of fetuin by hydrazinolysis (60°C, 6 hours) according to the kit protocol. Released O-glycans were labeled with 2-aminobenzamide using the Signal 2-AB Labeling Kit (product code GKK-404, available from ProZyme) and analyzed by normal-phase chromatography on the GlycoSep N column (product code GKI-4728, available from ProZyme) with fluorescence detection (ex 330 nm, em 420 nm) [Figure 2]. The profile of released O-glycans was consistent with the reported analysis of this glycoprotein (Merry *et al*., 2002 and Royle *et al*., 2002).

Peak	Structure
A	NeuAc α(2-3) Gal-2AB (degradation product)
B	NeuAc α(2-3) Gal β(1-3) GalNAc-2AB
C	Gal β(1-3) [NeuAc α(2-6)] GalNAc-2AB
D	NeuAc α(2-3) Gal β(1-3) [NeuAc α(2-6)] GalNAc-2AB
E	NeuAc α(2-3) Gal β(1-4) GlcNAc β(1-6) [NeuAc α(2-3) Gal β(1-3)] GalNAc-2AB

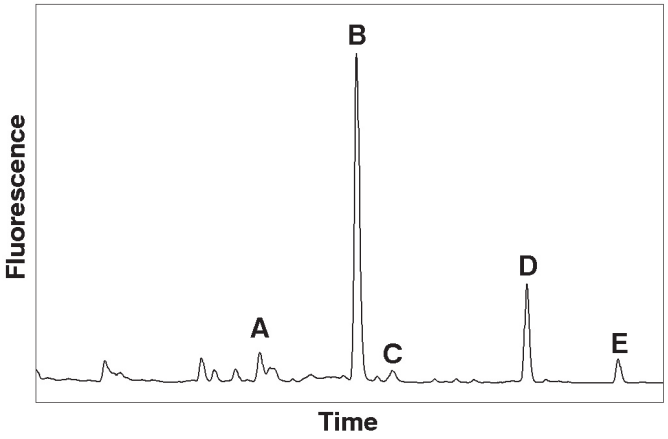

Figure 2 - HPLC Analysis of 2-AB-labeled O-Glycans Released from Fetuin by Hydrazinolysis

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APPENDIX A: TIPS, HINTS & TROUBLE SHOOTING

This kit is designed to be efficient and robust. If problems do arise, they can normally be corrected without difficulty. Here is a guide to the most likely problems, possible causes and solutions.

Degraded Glycans

The hydrazinolysis reaction mixture has been contaminated with moisture.

Moisture will adversely affect the hydrazinolysis reaction causing unwanted side reactions (peeling). Ensure that the sample is thoroughly lyophilized and that all glassware, syringes, *etc*. used to handle hydrazine are completely dry.

The sample contains salts, metal ions and/or detergents.

Samples must be free of these contaminants to avoid interference with the hydrazinolysis reaction.

Low Recovery of Glycans

The hydrazinolysis reaction mixture has been contaminated with moisture.

Moisture will adversely affect the hydrazinolysis reaction causing unwanted side reactions (peeling). Ensure that the sample is thoroughly lyophilized and that all glassware, syringes, *etc*. used to handle hydrazine are completely dry.

Desialylated Glycans

The sample has been exposed to low pH or high temperatures.

Desialylation can occur when samples are exposed to condition of low pH and/or high temperatures. Ensure that released glycan samples are dried as soon as possible after the Resin step (spin filtration) and are stored frozen. Avoid repetitive freeze-thaw cycles.

TECHNICAL ASSISTANCE

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

TOLL FREE **(800) 457-9444** (US & CANADA)
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ProZyme values customers opinions and considers customers an important source for information regarding advanced or specialized uses of our products. We encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.

Also, contact your local distributor:

http://www.prozyme.com/distributors.html

PRODUCT USE AND WARRANTY

Terms and conditions of sale as well as product warranties may be found at:

http://www.prozyme.com/terms.html

OTHER PROZYME PRODUCTS & KITS

ProZyme offers an Enzymatic Deglycosylation Kit (product code GK801110/GK80115) as well as a wide variety of exo-glycosidases that may be used in conjunction with hydrazinolysis.

A complete listing is available on our website:

http://www.prozyme.com

TRADEMARKS

ORDERING INFORMATION

For North American destinations: telephone orders may be placed between 8:00 am and 5:00 pm Pacific Time. Telefax or e-mail orders may be sent or messages recorded anytime.

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WEB **www.prozyme.com**

Outside North America:

A list of ProZyme's distributors, with contact information, may be found at:

http://www.prozyme.com/distributors.html

If there is no distributor in your area, place an international order directly at:

http://www.prozyme.com/ordering.html#outside_america

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