



GLYCOFREE™ CHEMICAL DEGLYCOSYLATION KIT

Rapid, convenient and near-complete deglycosylation of up to 24 mg of glycoconjugates (glycoproteins and glycopeptides) with anhydrous trifluoromethanesulfonic acid (TFMS).

- Leaves the primary structure of the protein intact
- Polypeptide recovery typically 90%
- Polypeptide cleavage <10%
- Extent of deglycosylation >80% (both O- and N-linked glycans on glycoproteins, proteoglycans and mucins)
- Up to 24 individual samples (2 batches of 12)

Product Code GKK-500

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Storage Conditions

Store at room temperature upon receipt.

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

KIT CONTENTS

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

Item	Qty
Reagent A TFMS (trifluoromethanesulfonic acid)	2 ea
Reagent B Toluene	2 ea
Reagent C Pyridine Solution (pyridine/methanol/water in a 3:1:1 ratio)	4 ea
Reaction vials, caps and septa (packaged in separate bags)	24 ea
Neutralization Solution (0.5% [w/v] ammonium bicarbonate)	~30 ml

Additional Required Reagents/Equipment

Dry ice/ethanol bath

Glass syringe gas-tight with Teflon® plunger [Hamilton #81101], fitted with a stainless steel needle (50 - 200 μ l volume [Hamilton #90022])

Lyophilizer

Desiccator with suitable drying agent

SAFETY AND HANDLING

Please read the Material Safety Data Sheets (MSDS) included in the package.

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.

Reagent A (TFMS) is a very powerful acid and should be handled in a fume hood while wearing eye protection and gloves. It is very hygroscopic. A fresh ampule of TFMS 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 5 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 reagents must be performed in a dry environment with dry glassware and plasticware.

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

INTRODUCTION

The presence of carbohydrates can often complicate physical and chemical characterization of the polypeptide moiety of a glycoprotein. Removal of the carbohydrate (while leaving the polypeptide intact) facilitates analysis of the protein, often revealing additional information about the polypeptide which may not be possible when the glycans are attached. For example, comparative analysis before and after deglycosylation allows determination of the relative contributions of carbohydrates to the molecular size of the protein, or the glycan contribution to the number of protein electrophoretic forms. In addition, deglycosylation can be used to obtain information regarding the functional significance of the carbohydrates on the protein.

Applications of glycoprotein deglycosylation have included:

- Determining the extent of glycosylation of a glycoprotein, giving a more accurate measure of the molecular weight of the polypeptide moiety
- Preparing glycopeptides and polypeptides for primary structural analysis
- Studying the functional role of glycans and properties conferred on glycoproteins
- Simplifying proteomic analysis of glycoproteins by minimizing multiple electrophoretic forms due to carbohydrate charge diversity

- Facilitating peptide mapping of protease-resistant proteins
- Confirming the O-linked glycan contribution to protein molecular size when used in conjunction with deglycosylation by enzymes which cleave only N-linked, but not O-linked, structures intact, *e.g.* N-Glycanase®
- Deglycosylation of proteoglycans and mucins

(Trimble and Maley, 1977; Leavitt *et al.*, 1977; Sojar and Bahl, 1987; Raju and Davidson, 1994; Frysedale *et al.*, 2002 and Edge, 2003)

Deglycosylation Methods

Deglycosylation of glycoproteins can be achieved by either enzymatic or chemical means. Both protocols have their advantages as well as their limitations. In some cases, it may be beneficial to use a combination of both (Raju and Davidson, 1994).

Chemical Deglycosylation with TFMS

In general, chemical deglycosylation with trifluoromethanesulfonic acid (TFMS) is non-specific and somewhat harsh in its action. Its primary value is that the acid treatment removes nearly all types of glycans from a wide variety of glycoproteins, mucins and proteoglycans.

Although the polypeptide chain of glycoconjugates is largely unaffected by TFMS treatment, carefully controlled deglycosylation conditions are required to maintain the protein

integrity. Low reaction temperatures, short time periods and strictly anhydrous conditions are absolutely necessary, making application of the method somewhat involved. Conditions have been optimized to give minimal alterations, if any, in the protein; however, not all proteins or enzymes may remain completely inert with this treatment.

A second disadvantage of the TFMS method is that it results in complete degradation of the glycan chains themselves. Thus, structural and compositional information about the glycans cannot be obtained, unlike enzymatic deglycosylation, which allows the recovery of both the polypeptide and the oligosaccharide portions.

The protocol presented here is based on a modification of the method described by Sojar and Bahl (1987). Its chief advantage over other chemical methods is the relatively mild conditions, resulting in minimal perturbation of the protein structure and amino acid composition (Edge, 2003).

TFMS, used under the conditions described herein, specifically cleaves the glycosidic bonds between carbohydrate moieties. Most glycosidic bonds are labile to TFMS cleavage; the susceptibility to cleavage varies with the nature of the sugars and their substituents. It should be noted that sugars directly linked to amino acids on the polypeptides, such as GlcNAc-Asparagine, GalNAc-Serine/Threonine or other monosaccharides covalently linked to Serine/Threonine are usually **NOT** cleaved under these conditions and the sugars

will be retained on the protein after TFMS treatment. A protocol has been included for mucins (page 21), which require additional processing to fully deglycosylate.

While little mechanistic information is available regarding the means by which TFMS deglycosylates glycoproteins, it is known that the reaction only occurs under anhydrous conditions, suggesting that deglycosylation may be achieved via an acid-catalyzed dehydration rather than a hydrolytic pathway. Thus, the amide bonds in the protein are stable to TFMS cleavage **under the conditions used to remove sugars**. Specifically, the sample must be highly dried, as any water allows the TFMS to hydrolyze the peptide bonds and degrade the protein.

Toluene is included here as a free-radical scavenger so that amino acid side chains are recovered intact; other scavengers, such as anisole or phenol have also been used. Scavengers provide a sink for reactive free radicals so the protein side chain residues are protected. In their absence, alteration of various amino acids has been described (Edge, 2003).

After the deglycosylation reaction is complete, excess TFMS is neutralized, resulting in the formation of a pyridinium salt. The salt is then removed yielding protein samples ready for analysis.

The protocol has been optimized with respect to reaction temperature, incubation time, choice of scavenger and reagent quantities, using such glycoproteins as bovine serum fetuin, human α 1-acid glycoprotein, horseradish peroxidase, hen egg ovalbumin and bovine pancreatic ribonuclease B.

Nevertheless, some glycoproteins may be unexpectedly sensitive or resistant to deglycosylation by TFMS. In such cases, a time-course analysis of the extent of deglycosylation may be required.

Other Chemical Methods

Other chemical methods for removing glycans from glycoproteins have been developed:

- Deglycosylation with gaseous hydrogen fluoride (Axelsson *et al.*, 1998)
- Hydrazinolysis (Patel and Parekh, 1994)
- Base-catalyzed reductive and non-reductive beta-elimination (Robbe *et al.*, 2003; Wilson *et al.*, 2002).

The latter two methods are used with the object of releasing glycans for further analysis rather than to study the protein.

Enzymatic Deglycosylation

Enzymatic deglycosylation is a more specific and gentle method of glycan removal. In contrast to TFMS, enzymatic deglycosylation is highly specific for the types of glycan structures that can be released, but no single enzyme has been

identified which cleaves all types of glycans. Specifically, no enzyme has yet been identified that can cleave large O-linked structures, such as those found on mucins or other highly substituted proteins, leaving them intact.

N-linked structures can be cleaved with *endo*-glycohydrolases such as N-Glycanase, or individual sugars can be specifically removed with a variety of *exo*-glycosidases. In the former case, the glycan structures are recovered intact for further analysis by chromatographic or spectral methods along with the protein, one of the major advantages of enzymatic cleavage.

A second advantage, particularly when N-Glycanase treatment is employed, is that the entire glycan moiety is removed from the polypeptide and available for analysis.

Enzymatic deglycosylation presents some disadvantages to counter the benefits:

- Extended reaction times may be needed to give complete glycan release.
- Some enzymes have a high degree of substrate, and even linkage, specificity.

Small modifications of the glycan, such as sulfation, phosphorylation or acetylation, can inhibit enzyme action. This is of particular concern when a previously un-characterized glycoprotein is analyzed.

- Inherent substrate specificities of glycohydrolases may

limit their action.

At present, no endoglycosidase has been identified that cleaves intact O-linked glycan structures from the polypeptide chain. Thus, enzymatic removal of O-linked carbohydrates can be a tedious procedure, requiring sequential treatment with a variety of exoglycosidases.

- All glycan or sugar moieties may not be accessible to a specific glycohydrolase because of conformation or structural restraints imposed by the substrate glycoprotein.

In many cases denaturation of the glycoprotein is required in order to expose the glycans to the *endo*- or *exo*-glycosidase action to give complete glycan removal. In this case, unlike TFMS treatment, the structural integrity of the glycoprotein is not maintained under reducing conditions in the presence of strong detergents.

Using the Kit

Kit reagents are shipped in ampules surrounded by vermiculite and sealed in an aluminum can. Take care when unpacking as the ampules are fragile.

A fresh ampule of Reagent A (TFMS) must be used for each batch of samples; the reagent should be discarded after exposure to atmospheric moisture for longer than 5 minutes. Two sets of reagents have been included to allow more

flexibility for the user (a set consists of 1 ampule each of Reagent A and B, and 2 each of Reagent C). Up to 12 samples may be deglycosylated with one set of reagents.

An ethanol/dry ice bath is needed prior to addition of reagents and during the neutralization step.

Opening the Component Ampules

Make sure to take proper safety measures for handling the contents as described in the enclosed MSDS's. Gently tap the ampule to settle the contents on the bottom. The Reagent A (TFMS) ampule must be scored prior to opening; the others have pre-scored necks. To open, hold both the body and the top of the ampule, then gently but firmly snap open at the colored break-ring. Snap away from your body. Be careful of the sharp edges of the opening (*be sure to wear gloves and safety glasses during these operations*).

Fluids (particularly TFMS) should be pipetted with chemically inert syringes. After the reaction is complete and the samples have been neutralized, manual pipettors with plastic tips can be used for liquid transfer.

Assembling the Reaction Vials

The reaction vials, caps and septa are supplied un-assembled for convenience in sample preparation. Please note that the septa have one side that is Teflon[®]-coated. It is important that the septum be inserted into the cap with the Teflon surface facing toward the vial.

Using the Syringe with the Reaction Vials

The TFMS/Toluene mixture should be transferred 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 dry, reagent-grade acetone, flush with a stream of dry nitrogen, warm in a dry oven (~90°C) and 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: Plastic disposable 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 TMSF.

When piercing the septum with the syringe needle for reagent addition, precautions should be taken to avoid personal injury. The use of a remote handling device is recommended to hold the vial during needle insertion. Alternatively, impenetrable hand or finger protection should be worn.

PROTOCOLS

Outline of the Deglycosylation Procedure

1. Sample preparation

Glycoproteins or glycopeptides should be relatively free of salts, metal ions and detergents, thoroughly dried in the reaction vials and sealed with the caps and septa provided.

2. Reagent preparation

Reagents are prepared fresh and used within 5 minutes.

3. Deglycosylation reaction

The reaction is carried out in an ethanol/dry ice bath, followed by incubation at -20°C for 4 hours.

4. Reaction termination

Excess TFMS is neutralized by addition of Reagent B and Reagent C.

5. Protein recovery/separation of reaction by-products

Reaction products and carbohydrate derivatives are separated from the polypeptide by exploiting molecular size differences, *e.g.* dialysis, size exclusion chromatography, *etc.*

6. Analysis of the protein/peptide

The deglycosylated polypeptide is ready for analysis, e.g. SDS-PAGE, 2-D gel electrophoresis, structural analysis, functional assays, immunology studies, *etc.*

Sample Preparation

Reagents and components

Samples - solutions of glycoproteins or glycopeptides relatively free of salts, metal ions and detergents. Sample quantities should be in the range of 0.25 - 1 mg, depending on the extent of glycosylation.

NOTE: An acceptable sample may be prepared by either dialyzing against an appropriate solvent (e.g. 0.1% [v/v] trifluoroacetic acid), or gel filtration or reverse phase HPLC in a volatile solvent system.

NOTE: The TFMS reaction tolerates small amounts of SDS in the sample. Since this may be sample dependent, SDS should be kept to a minimum.

Reaction vials, caps and Teflon-faced seals (supplied with the kit)

NOTE: Leave reaction vials, caps and septa wrapped until ready for use to avoid contamination. In a humid environment, they may be stored in a desiccator (with bags open).

Procedure

Transfer the sample solution (≤ 0.5 ml) to the bottom of a reaction vial and freeze-dry thoroughly. Tightly seal the vial using the Teflon-lined septum and cap. Store in a desiccator prior to initiating the deglycosylation reaction.

NOTE: The samples must be thoroughly dry prior to reagent addition to avoid hydrolysis of the peptide bonds. Ensure that the drying step reaches completion and that the dehydrated samples are not exposed to moisture before the reaction. Lyophilization of samples is recommended (≤ 50 milliTorr; >24 hours).

Ensure that the sample dries to a compact mass at the very bottom of the tube.

Deglycosylation Reaction

Reagents and Supplies

Dry ice/ethanol bath

NOTE: Prepare dry ice/ethanol bath by adding pieces of dry ice to a suitable dish, followed by the slow addition of ethanol. Add just enough alcohol to cover the ice pieces.

Reagent A (TFMS, supplied with the kit)

Reagent B (Toluene, supplied with the kit)

Gas-tight, Teflon-tipped plunger, glass syringe and stainless steel needle (clean and thoroughly dried)

Procedure

Score the Reagent A (TFMS) ampule. Tap Reagent A and Reagent B gently to ensure the contents are at the bottom. Break open the ampules.

Using the (dry) syringe, add 60 μl of Reagent B to Reagent A. Shake gently to mix. Rinse the syringe once with the TFMS/Toluene mixture.

Place samples into the dry ice/ethanol bath so that they rest firmly on the dry ice and against the side of the vessel. Allow samples to cool in the bath for approximately 20 seconds.

Use the syringe to add 50 μl of the TFMS/Toluene mixture to the first sample by piercing the Teflon-lined septum of the vial with the syringe needle. Withdraw the needle and leave the sample vial in the bath while processing the rest of the samples.

NOTE: Allow the TFMS/Toluene mixture to run slowly down the side of the reaction vessel over a period of 15 - 20 seconds. Do not be concerned if some of the reagent freezes on the side of the reaction vessel.

Place vials in a freezer at -20°C . After 5 minutes, shake briefly to aid melting of the contents and subsequent solvation of the glycoprotein.

Replace in the freezer for another 5 minutes, then shake again.

NOTE: The contents of the vial should have melted and appear homogeneous.

Place vial in the freezer for 4 hours.

Neutralization of Excess TFMS

Reagents and Supplies

Dry ice/ethanol bath

Reagent C (Pyridine Solution, supplied with the kit)

Dry ice

Wet ice

Neutralization Solution (supplied with the kit)

Procedure

Remove the vials from the freezer and remove the caps and the septa from the vials.

NOTE: The neutralization reaction is highly exothermic. Removal of the cap allows venting of gases that may be released during this process.

Place the vials in the ethanol/dry ice bath and allow to cool for 20 seconds.

Using a micropipette, slowly add 150 μl of Reagent C to each vial. Allow the solution to flow down the side of the vial over a period of 15-20 seconds. Leave the vial in the bath while processing the rest of the samples, or for a minimum of 20 seconds.

Transfer the vial to dry ice for 5 minutes and then to wet ice for a further 15 minutes.

Add 400 μl of Neutralization Solution to the vial and mix briefly.

NOTE: At this point the polypeptide may form a precipitate. The choice of recovery method should be chosen to minimize sample losses.

Recovery of Deglycosylated Polypeptide

Precipitation

In cases where precipitation is observed upon neutralization, it may be possible to isolate the precipitated polypeptide directly by centrifugation:

Cool the sample to 4°C and allow to stand at this temperature for 30 minutes.

Centrifuge at high speed for 10 - 15 minutes at 4°C to sediment precipitated material.

Decant and retain the supernatant (in case of incomplete precipitation). Resuspend the pellet in a small volume of ice cold buffer and re-centrifuge at 4°C.

Resuspend and centrifuge at least three more times.

Gel Filtration

In cases where no precipitation of the peptide is observed, the polypeptide may be isolated by passing it through a small gel filtration column. Commercially available gel filtration columns can be chosen based on the molecular weight of the polypeptide.

Dialysis

Transfer each sample to a dialysis cassette, bag or, preferably, a flow-dialysis apparatus. If batch dialysis is used, dialyze against a minimum of 3 x 1000 volumes of 10 mM ammonium carbonate. A minimum of 2 hours dialysis time is recommended for each batch. If flow dialysis is used, dialyze against 500 - 1000 volumes of buffer over a period of 8 hours.

Deglycosylation of Mucins

The removal of glycans from mucins is more difficult than from glycoproteins. The high density of sialic acid residues on the polypeptide can limit the degree of deglycosylation with TFMS treatment. Thus, it has been suggested that the mucin be treated first with sialidase to enzymatically remove sialic acids followed by TFMS hydrolysis.

In addition, the innermost GalNAc often proves to be unaffected by TFMS treatment. One method which has proved successful in removing the innermost GalNAc is described here (customer report), which is a modification of a method previously described (Gerken *et al.*, 1992 and Raju and Davidson, 1994).

Reagents

Dialysis Buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.5)

Sodium Periodate Solution (100 mM stock solution)

Sialidase A™ (Product Code GK80040, an $\alpha(2-3,6,8,9)$ -specific sialidase, available from ProZyme)

Procedure

Treat the sample glycoprotein (~1 mg) with Sialidase A using the instructions and reaction buffer supplied with the enzyme.

Prepare the desialylated sample by dialysis against water and dry the resulting protein.

Deglycosylate as described in the standard protocol (page 13).

After neutralization, dialyze the sample against Dialysis Buffer.

Add Sodium Periodate Solution to a 10 mM final concentration in the sample.

Incubate for 6 hours at 4°C.

Dialyze against water and, for the second time, prepare and deglycosylate the sample as described in the standard protocol (page 13).

REFERENCES

- Axelsson, M. A., Hansson, E. M., Sikut, R. and G. C. Hansson. **Glycoconjugate J** **15**, 749-755 (1998).
- Edge, A. S. **Biochem J** **376**, 339-350 (2003).
- Frysdale, B. G., Jedrzejewski, P. T., Wong, D. L. *et al.* **Electrophoresis** **23**, 2184-2193 (2002).
- Gerken, T. A., Gupta, R. and N. Jectoft. **Biochemistry** **31**, 639-649 (1992).
- Leavitt, R., Schlesinger, S. and S. Kornfield. **J Virol** **11**, 75 (1977).
- Patel, T. P. and R. B. Parekh. **Meth Enzymol** **230**, 57-66 (1994).
- Robbe, C., Capon, C., Flahaut, C. and J. C. Michalski. **Electrophoresis** **24**, 611-621 (2003).
- Raju, S. and E. A. Davidson. **Biochem and Mol Biol Intl** **34**, 943-954 (1994).
- Sojar, H.T. and O.P. Bahl. **Meth Enzymol** **138**, 314 (1987).
- Trimble, R.B. and F. Maley. **J Biol Chem** **252**, 4409 (1977).
- Wilson, N. L., Schulz, B. L., Karlsson, N. G. and N. H. Packer. **J Proteome Res** **1**, 521-529 (2002).

APPENDIX A: TIPS, HINTS & TROUBLE SHOOTING

Deglycosylation is Incomplete

The sample may not have been sufficiently dry prior to deglycosylation. Ensure that the sample is lyophilized for a minimum of 24 hours (≤ 50 milliTorr); avoid extended exposure to atmospheric moisture between drying and deglycosylation stages. It is highly recommended that the reagents, vials, caps, septa (open the bags) and syringe be stored in a desiccator prior to use.

NOTE: In cases where humidity is high, it may be necessary to perform the entire reaction in a dry box.

The sample may have contained inhibiting levels of salts, metal ions or detergents. A control glycoprotein may be included in the sample set that is formulated the same as the protein of interest. The efficiency of deglycosylation can be evaluated (SDS-PAGE) under these conditions using the control protein as an indicator.

The deglycosylation reagents may have been exposed to the atmosphere too long prior to addition. Ensure that all samples are conveniently placed so that reagent addition to successive samples can be completed within 5 minutes of opening the individual reagent ampules.

Sample and reagents may not have been mixed sufficiently during the incubation at -20°C . On inspection of the vial at 5 and 10 minutes, shake well and swirl the liquid contents around the sides of the vessel until all solid material is washed to the bottom.

The particular glycoprotein may be resistant to deglycosylation by TFMS under the conditions used. A time-course analysis of the extent of deglycosylation can be performed. If so, it is suggested that the temperature be maintained at -20°C . An extended reaction time of up to 4-8 hours may be suitable.

Peptide Damage Apparent on Analysis after Deglycosylation

Sample may not have been sufficiently cooled prior to TFMS addition or neutralization and the reaction was overheated. Ensure that at least one third of the reaction vessel is immersed in a dry ice/ethanol bath for a minimum of 20 seconds prior to **slow** addition of TFMS/Toluene mixture.

Anhydrous conditions may not have been maintained, so peptide bond cleavage may have occurred due to hydrolysis (see suggestions under "Deglycosylation is Incomplete").

Low Recovery of Polypeptide

Precipitation – The protein may have partially dissolved during the washing procedure on the pelleted reaction products. Retain and pool all washings plus the supernatant from the initial centrifugation. Evaporate and reconstitute with buffer. Isolate remaining polypeptide from this solution using dialysis or gel filtration.

Resuspending the precipitated protein in an organic solvent (acetonitrile, butanol, *etc.*) or buffer with detergent may assist in solubilizing the deglycosylated protein.

Gel Filtration – Polypeptide may have precipitated while passing through the gel filtration column. Use one of the alternative methods for polypeptide recovery.

Dialysis – Material may not have been completely recovered from the dialysis chamber. Rinse the dialysis chamber at least twice with buffer after dialysis is complete. This is particularly important if signs of precipitation are apparent during dialysis.

TECHNICAL ASSISTANCE

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

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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:

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OTHER PROZYME PRODUCTS & KITS

ProZyme offers an Enzymatic Deglycosylation Kit (product code GK801110/GK80115) as well as a wide variety of exoglycosidases that may be used in conjunction with chemical deglycosylation.

A complete listing is accessible on our website:

<http://www.prozyme.com/glyko/enzymes.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>

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