

POST-TRANSLATIONAL MODIFICATIONS



GlycoProfile™ I Enzymatic In-gel N-Deglycosylation Kit

Glycosylation often leads to problems in subsequent protein analysis procedures. Glycopeptides generally do not readily ionize during MS analysis leading to insufficient spectral data. Furthermore, proteolytic digestion of the native glycoprotein is often incomplete due to steric hindrance by the oligosaccharides. Removal of the carbohydrate groups from a glycoprotein prior to protein identification is preferred.

Sigma's GlycoProfile I Enzymatic In-gel N-Deglycosylation Kit is optimized to provide a convenient and reproducible method to N-deglycosylate and tryptically digest protein samples in 1D or 2D polyacrylamide gel slices for subsequent MS or HPLC analysis. The procedure is suitable for Coomassie Blue and Colloidal Coomassie stained gels and may be used with gels silver stained and destained using Sigma's Proteo Silver™ Plus kit ([PROT-SIL2](#)). GlycoProfile Enzymatic In-gel N-Deglycosylation kit includes the enzymes and reagents necessary for N-linked deglycosylation and tryptic digestion. The samples can then be desalted and concentrated for analysis by MALDI-TOF or electrospray MS.

Features & Benefits

- **Provides all components for in-gel deglycosylation and trypsinization of protein samples** – Conveniently prepares deglycosylated protein samples for analysis by MS or HPLC
- **Utilizes PNGase F for the enzymatic removal of N-linked glycans** – Proteins remain intact, unlike the use of chemical deglycosylation which can degrade the protein
- **Includes Proteomics Grade PNGase F and Trypsin** – Highly purified enzymes possess no unwanted activities or additives to complicate analysis
- **PNGase F is supplied lyophilized from a low salt buffer** – Allows reconstitution of the enzyme to any concentration needed
- **Works in solution or with gel slices** – Allows choice of methods

Components

Destaining Solution

Proteomics Grade PNGase F

Proteomics Grade Trypsin

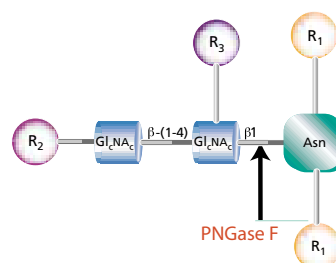
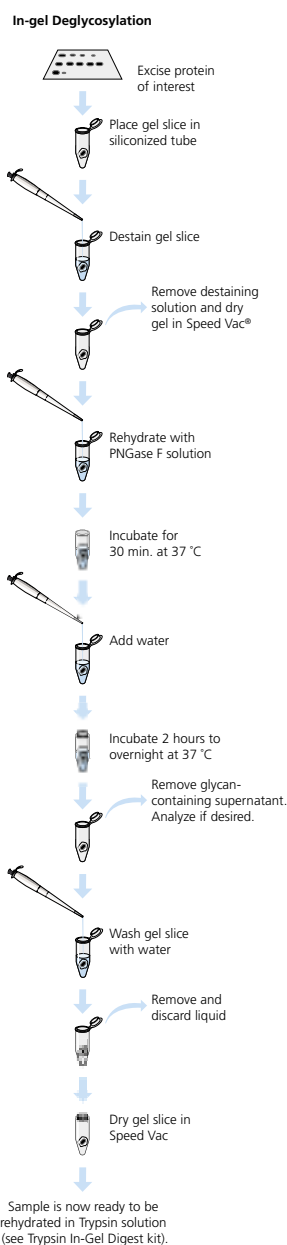
Trypsin Solubilization Reagent

Trypsin Reaction Buffer

Invertase (positive control)

Peptide Extraction Solution

Biotech Grade Acetonitrile



R₁ = N and C substitution by groups other than H
 R₂ = H or the rest of an oligosaccharide
 R₃ = H or α 1,6 fucose

Product Code	Description	Size
PP0200	GlycoProfile I	1 kit

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GlycoProfile™ II

Enzymatic In Solution N-Deglycosylation Kit

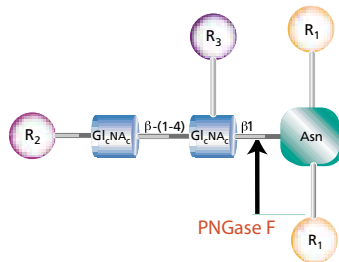
The GlycoProfile Enzymatic In-Solution N-Deglycosylation Kit has been optimized to provide a convenient and reproducible method to remove N-linked glycans from glycoproteins and is compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from any of the reaction components. The kit contains sufficient enzyme, glycoprotein standard and reagents, for a minimum of 20 reactions when the sample size is between one to two mg of a typical glycoprotein.

Features & Benefits

- **Provides all components for in-solution N-linked deglycosylation of protein samples.** – Conveniently prepares deglycosylated protein samples for analysis by MS, HPLC and PAGE
- **Reagents are optimized for direct MS analysis** – No need for post-reaction sample clean up
- **Utilizes PNGase F for the enzymatic removal of N-linked glycans** – Proteins remain intact, unlike the use of chemical deglycosylation which can degrade the protein
- **Includes Proteomics Grade PNGase F and Trypsin** – Highly purified enzymes possess no unwanted activities or additives to complicate analysis
- **PNGase F is supplied lyophilized from a low salt buffer** – Allows reconstitution of the enzyme to any concentration needed

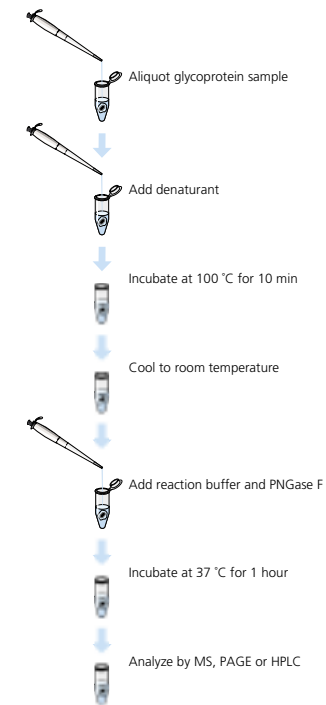
Components

Proteomics Grade PNGase F
Ribonuclease B
10x Reaction Buffer
Octyl β-D-Glucopyranoside
2-Mercaptoethanol



R₁ = N and C substitution by groups other than H
 R₂ = H or the rest of an oligosaccharide
 R₃ = H or α,1,6 fucose

In-solution Deglycosylation



Product Code	Description	Size
PP0201	GlycoProfile II	1 kit

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In contrast to the GlycoProfile™ In-gel Deglycosylation Kit, the following kits are optimized for the removal of glycans from proteins in solution. This can simplify electrophoretic analysis by reducing the heterogeneity normally associated with glycoproteins. The kits are also useful if the protein(s) of interest are not separated by electrophoresis but analyzed by other means.

Components

PNGase F
Endo-O-Glycosidase
$\alpha(2\rightarrow3,6,8,9)$ -Neuraminidase (Sialidase A)
$\beta(1\rightarrow4)$ -Galactosidase
β -N-Acetylglucosaminidase
5x Reaction Buffer
Denaturation Solution
TRITON™ X-100 (15% solution)
Fetuin Control

Enzymatic Protein Deglycosylation Kit

The E-DEGLY Kit contains the enzymes and reagents required for deglycosylation of both N- and O-linked glycans.

Features & Benefits

- **Single reaction at neutral pH** – *No need to adjust conditions for each glycosidase reaction*
- **Native & denaturing procedures** – *Allows flexibility to ensure resulting deglycosylated proteins are compatible with down-stream analysis*
- **Uses highly purified glycosidases, free of contaminating proteases** – *No degradation of protein results in clear, reliable results*
- **Includes fetuin which has sialylated N- and O-linked oligosaccharides** – *Provides a control to check for efficiency of the deglycosylation*
- **Each reaction deglycosylates 200 μ g of glycoprotein** – *Yields enough deglycosylated protein for further analysis*
- **Contains sufficient reagent for the deglycosylation of approx. 2 mg of protein** – *Allows for the processing of multiple samples*

Product Code	Description	Size
E-DEGLY	Enzymatic Protein Deglycosylation Kit	1 kit

Components

Endoglycosidase F1, F2 and F3
Endoglycosidase F1 Reaction Buffer
Endoglycosidase F2 and F3 Reaction Buffer

Native Protein Deglycosylation Kit

The N-DEGLY Kit is intended for the deglycosylation of N-linked oligosaccharides from glycoproteins under native conditions. Particular residues, due to their location in the native protein, may be resistant to the traditional deglycosylation methods using PNGase F. The N-DEGLY Kit utilizes endoglycosidases which are less sensitive to protein conformation than PNGase F and, therefore, more suitable for deglycosylation of native proteins.

Features & Benefits

- **Enzymes included are less sensitive to protein conformation than PNGase F** – *Allows the deglycosylation of native proteins*
- **Uses highly purified glycosidases, free of contaminating proteases** – *No degradation of protein provides reliable, clear data*
- **Each reaction deglycosylates 200 μ g of glycoprotein** – *Yields enough deglycosylated protein for further analysis*
- **Contains sufficient reagent for the deglycosylation of approx. 2 mg of protein** – *Allows for the processing of multiple samples*

Product Code	Description	Size
N-DEGLY	Native Protein Deglycosylation Kit	1 kit

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Glycoprotein Detection Kit

Sigma's colorimetric glycoprotein detection kit enables convenient, reliable detection of the sugar moieties of glycoproteins in PAGE gels or Western blotting membranes. Based on a modification of Periodic acid-Schiff (PAS) methods, the Glycoprotein Detection kit identifies glycoproteins with magenta bands on a light pink or colorless background. The kit contains reagents sufficient to stain ten mini gels (8 x 10 cm) or ten membranes of the same dimensions and detects as little as 25 to 100 ng of carbohydrate, depending on the nature and degree of glycosylation.

Components

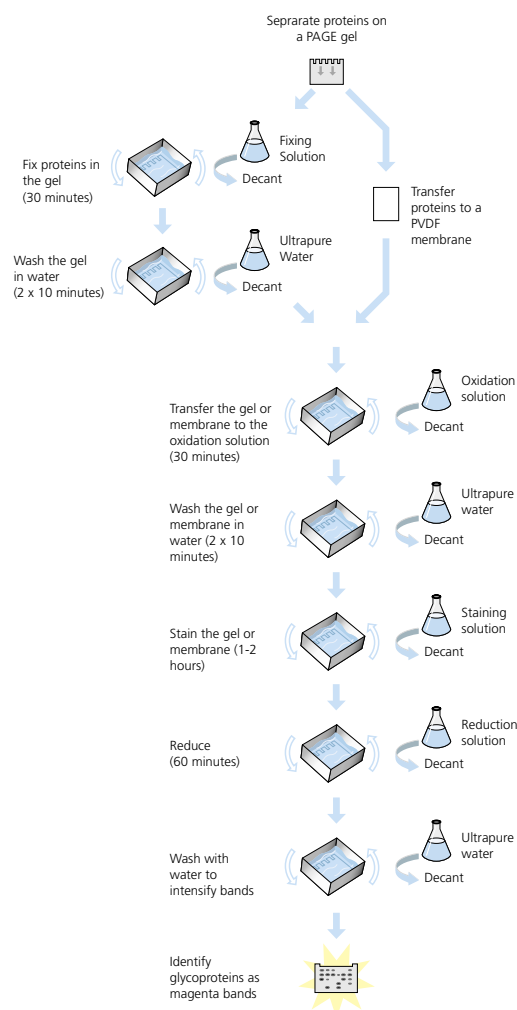
Oxidation Reagent (Periodic Acid)

Reduction Reagent (Sodium Metabisulfite)

Schiff's Reagent, Fuchsin-Sulfite Reagent

Horseradish Peroxidase Standard

Product Code	Description	Size
GLYCO-PRO	Glycoprotein Detection Kit	1 kit



Lectins and Other Products for the Detection of Glycoproteins

Product Code	Name	Use
A8_980-4	Anthranilamide (2-aminobenzamide)	Fluorescently labels glycans with a free reducing terminus; labeled glycan Ex=330 nm; Em=420 nm
A 1506	Anthranilic acid (2-aminobenzoic acid)	Fluorescently labels glycans with a free reducing terminus; labeled glycan Ex=315 nm; Em=400 nm
C 2272	Biotin labeled Concanavalin A	Detects glycoproteins containing α -D-mannose, α -D-glucose when used with avidin or streptavidin detection system
C 7642	FITC labeled Concanavalin A	Fluorescently labeled for the detection of glycoproteins containing α -D-mannose, α -D-glucose
L 6397	Peroxidase labeled Concanavalin A	Detects glycoproteins containing α -D-mannose, α -D-glucose when used with appropriate peroxidase substrate
L 5142	Biotin labeled lectin from <i>Triticum vulgare</i>	Detects glycoproteins containing β -(1,4)-GlcNac when used with avidin or streptavidin detection system
L 4895	FITC labeled lectin from <i>Triticum vulgare</i>	Fluorescently labeled for the detection of glycoproteins containing β -(1,4)-GlcNac
L 3892	Peroxidase labeled lectin from <i>Triticum vulgare</i>	Detects glycoproteins containing β -(1,4)-GlcNac when used with appropriate peroxidase substrate
M 5170	Mouse IgM λ (MOPC 104e)	Binds α -(1,3) linked glucose
P 0430	Periodic Acid	For selective oxidation of glycoproteins on SDS-PAGE gels and membranes
S 5133	Schiff's Reagent	Fuchsin-Sulfite reagent for detection of glycoproteins in polyacrylamide gels following periodate oxidation
D 0520	3-(Dansylamino)phenylboronic acid	For fluorescent labeling of <i>cis</i> -diols

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GlycoProfile™ III Fluorescent Glycoprotein Staining kit

Identify glycoproteins with superior selectivity using Sigma's new GlycoProfile™ III Detection Kit. Allows fluorescent detection of glycoproteins directly in PAGE gels without blotting or use of antibodies.

ProteoProfile PTM Marker (Product Code [P 1745](#)) contains a mix of glycosylated, phosphorylated and unmodified proteins and is available individually as well as with GlycoProfile III.

Features & Benefits

- **Superior Selectivity** – Enables more accurate detection of glycoproteins
- **Excellent Sensitivity** – Allows you to detect 150 ng of glycoprotein (5 to 30 ng) carbohydrate
- **Compatibility with both PAGE gels and PVDF membranes** – Allows flexibility in your applications

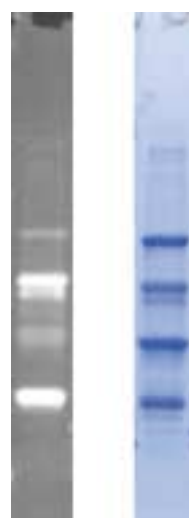
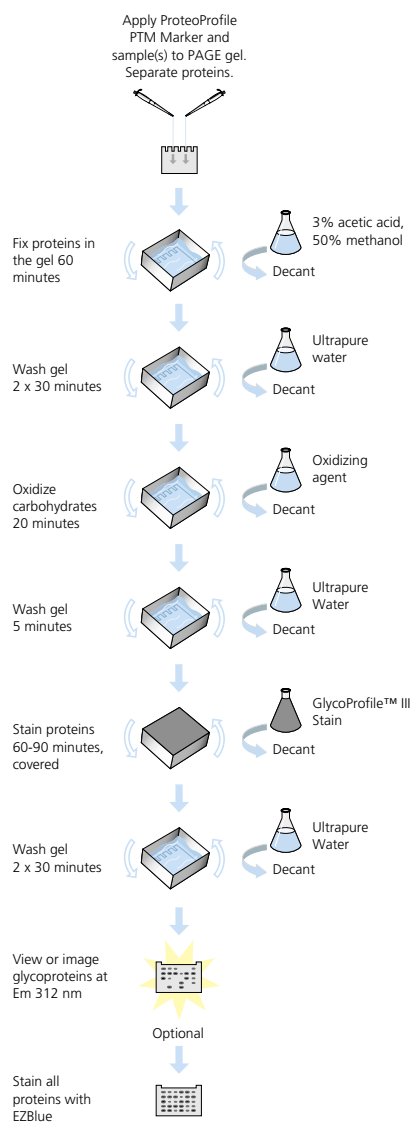
Components

ProteoProfile™ PTM Marker

Oxidation Reagent

Glycoprotein Staining Reagent

Wash Buffer



GlycoProfile III (PP0300) EZBlue™ (G 1041)

BSA (not glycosylated, not phosphorylated)
Ovalbumin (glycosylated and phosphorylated)
β-Casein (not glycosylated, phosphorylated)
RNase B (glycosylated, not phosphorylated)

Superior Selectivity ProteoProfile PTM Marker ([P 1745](#)), containing glycosylated and non-glycosylated proteins, was separated by electrophoresis on a 4-20% SDS-PAGE gel. The gel was stained with GlycoProfile III ([PP0300](#)) (left), fluorescently imaged, and then stained for total protein with EZBlue™ Gel staining reagent ([G 1041](#)) (right). Each band represents approximately 300 ng of protein.

Product Code	Description	Size
PP0300	GlycoProfile III	1 kit
P 1745	ProteoProfile PTM Marker	1 vial