

Glycoprotein Detection

Initial detection of glycoproteins *in vitro* is routinely accomplished on SDS-PAGE gels and Western blots. Cellular localization of glycoproteins is normally accomplished utilizing lectin fluorescent conjugates.

For a complete list of lectins including fluorescent labeled lectins, see page 80.

Colorimetric Detection on PAGE and Western blots

Glycoprotein Detection Kit

Product Code [GLYCO-PRO](#)

Product Description

The Glycoprotein Detection Kit provides a system to easily detect the sugar moieties of glycoproteins on SDS-PAGE or on Western blotting membranes. This detection system is a modification of Periodic acid-Schiff (PAS) methods and yields magenta bands with a light pink or colorless background. The detection limits have been found to be 25-100 ng of carbohydrates depending on the nature and the degree of glycosylation of proteins. Peroxidase

from horseradish, reported as having a carbohydrate content of approximately 16%, is used as a positive control in the kit. The table below describes the steps and time required when utilizing this kit.

Contains sufficient materials to stain 10 mini gels (8 x 10 cm) or 5 large gels (16 x 18 cm) or same sizes of blotting membranes.

Components

Oxidation Component (Periodic Acid)

Reduction Component (Sodium Metabisulfite)

Schiff's Reagent, Fuchsin-Sulfite Reagent

Peroxidase

Steps	Time for gel thickness 0.5-0.75 mm or for membrane	Time for gel thickness 1.0-1.5 mm
1. Fixing	30 min.	60 min.
2. Washing	2 x 10 min.	2 x 20 min.
3. Oxidation	30 min.	60 min.
4. Washing	2 x 10 min.	2 x 20 min.
5. Staining	1-2 hours or until bands turn magenta	1-2 hours or until bands turn magenta
6. Reduction	60 min.	120 min.
7. Washing	Band color will intensify with changes of fresh water	Band color will intensify with changes of fresh water
8. Storage	overnight	overnight

Glycoprotein Detection

Fluorescent Detection on PAGE Gels

GlycoProfile™ III Fluorescent Glycoprotein Detection Kit

NEW Product Code [PP0300](#)

Product Description

GlycoProfile™ III is designed for the fluorescent in-gel detection of glycosylated proteins utilizing standard UV-transillumination. Following SDS-PAGE, proteins are fixed in the gel with an acetic acid/methanol solution. The carbohydrates on the proteins are oxidized to aldehydes with periodic acid. A hydrazide dye is reacted with the aldehydes, forming a stable fluorescent conjugate. This allows for the specific, sensitive detection of the glycoproteins directly in gels. This kit can also be used to detect glycoproteins after Western transfer to PVDF membranes.

The classical method for in-gel carbohydrate detection uses Periodic Acid-Schiff reagent (PAS). It has a detection limit of 25-100 ng of carbohydrate. PAS staining of glycoproteins is very selective, but lacks the sensitivity of fluorescent detection (5-25 ng of carbohydrate). The limit of detection varies with the glycoprotein and the degree and type of glycosylation. Typical detection limits observed with the ProteoProfile PTM Marker (P 1745) are 150 ng of ovalbumin (5 ng of carbohydrate) and 150 ng of RNase B (30 ng carbohydrate). The detection limits are 5-10 times lower than those observed with the Periodic Acid-Schiff reagent.

Sufficient reagents are supplied for 10 mini gels.

Components

Oxidation Reagent

Glycoprotein Staining Reagent

Staining Buffer

ProteoProfile PTM Marker

Designed as both a positive and negative control for SDS-PAGE gels and Western blots of proteins with post-translation modifications.

Specificity

Although this staining procedure is quite selective for glycoproteins, some non-specific protein staining may occur and may be more pronounced in some gel formulations. Staining the gel with EZBlue™ Gel Staining Reagent after fluorescent imaging will allow identification of non-specifically stained proteins.

An alternative method is to run duplicate gels and fluorescently stain the second gel omitting the oxidation step. Any fluorescent staining will be non-specific.

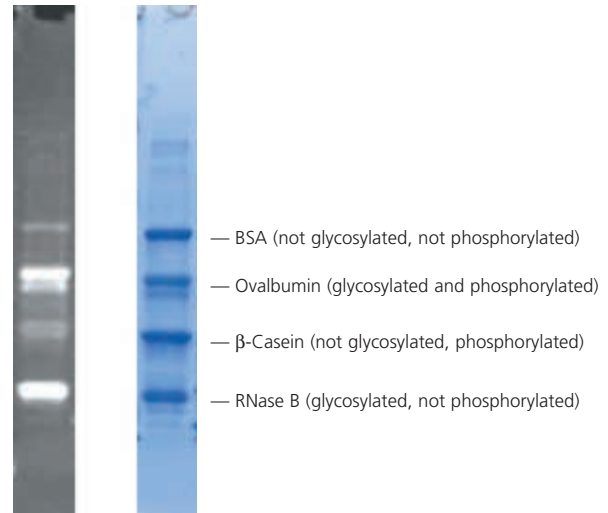


Figure 1. PTM Marker (2 μ l of a 6-fold dilution), containing glycosylated and non-glycosylated proteins, was separated by electrophoresis on a 4 \rightarrow 20% SDS-PAGE gel. The gel was stained for glycoproteins with GlycoProfile III (left), imaged, and then stained for total protein with EZBlue Gel Staining Reagent (right). The glycoproteins appear as bright fluorescent bands. Each band represents approximately 300 ng of protein.

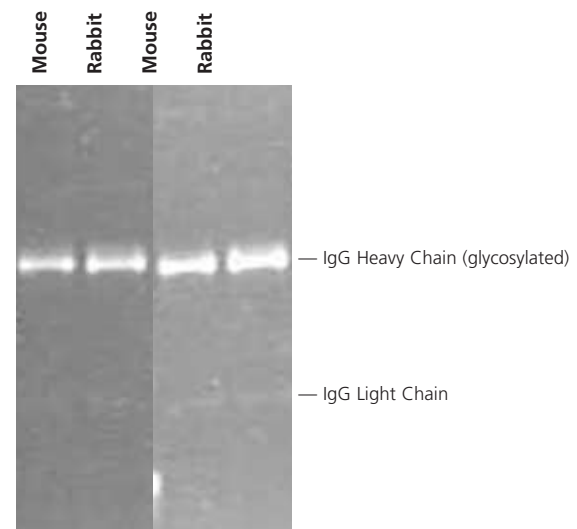


Figure 2. Mouse IgG and rabbit IgG were separated on a 4 \rightarrow 20% SDS-PAGE gel and stained in the same manner as the gel in Figure 1. The IgG heavy chains, which are glycosylated, react strongly with the fluorescent detection reagent. 2.5 μ g of protein was applied to each lane.

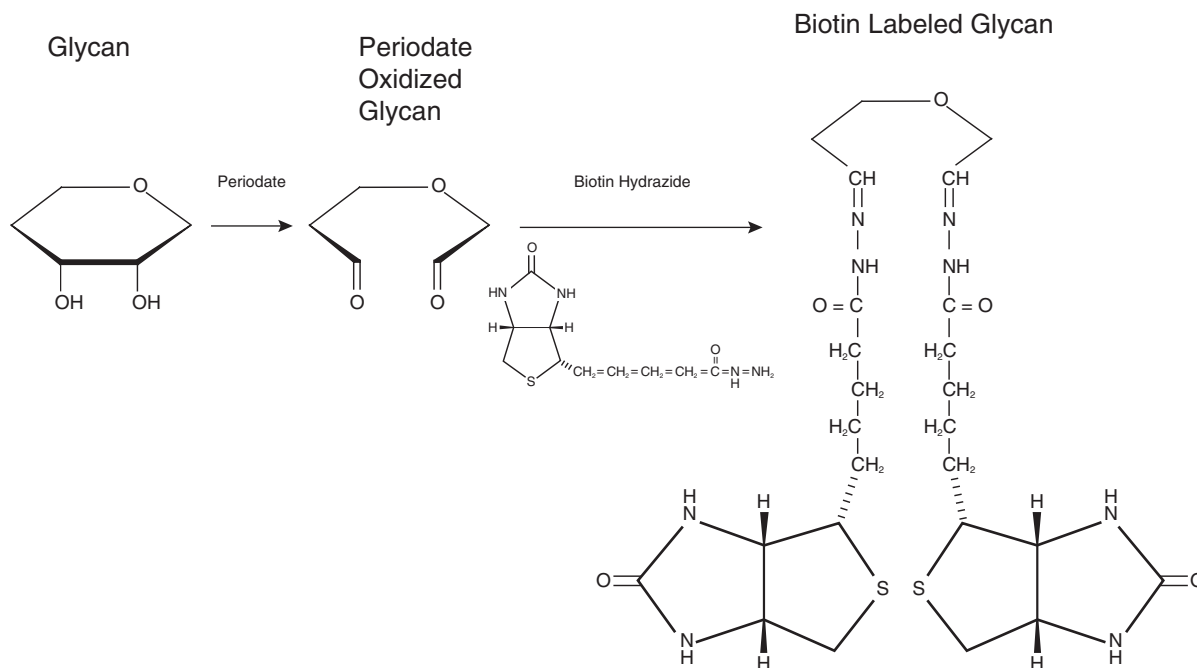
Glycoprotein Detection

Detection of Biotin Labeled Glycoproteins on Western blots

Biotin-hydrazide modification of periodate oxidized glycans can be utilized to label glycoproteins on Western blots. The blots can then be probed with streptavidin-peroxidase.¹ Detection can be accomplished with either

colorimetric TMB or chemiluminescent CPS-1 peroxidase substrate solutions. Alkaline phosphatase conjugated streptavidin can also be used to probe the blots. Typical detection limits for glycoproteins using this method are approximately 50-100 ng.

Optimal results are obtained on PDF membranes.



Detection Products

B 7639	Biotin Hydrazide
S 5512	Streptavidin, Peroxidase Labeled
S 1878	Sodium (meta) Periodate
T 0565	Tetramethylbenzidine Liquid Substrate System
CPS1-60	Chemiluminescent Peroxidase Substrate 60 ml
CPS1-120	Chemiluminescent Peroxidase Substrate 120 ml
CPS1-300	Chemiluminescent Peroxidase Substrate 300 ml

Reference

1. Bayer, E.A., et al., Meth. Enzymol., **184**, 415 (1990).

Glycoprotein Detection

Glycoproteins and Glycoprotein Standards

There are many methods currently utilized in glycoprotein detection and identification. Among the most common are SDS-PAGE, Mass Spectroscopy, HPLC, NMR, and Western blot. It is helpful and often necessary to include glycoproteins as standards and/or molecular weight markers.

In biological assays involving cell or animal models, the physiologic activity of glycoproteins is of primary interest. Sigma-Aldrich offers a broad variety of glycoproteins for analytical and biological use as standards, controls, and bioactive components. The following products are an abbreviated list of the most popular glycoproteins in common analytical and biological assays. For additional products please visit our website at sigma-aldrich.com.

Preferred Glycoprotein Standards

I 0408 NEW	Invertase Glycoprotein Standard	Invertase is an enzyme that catalyzes the hydrolysis of sucrose into fructose and glucose. Invertase Glycoprotein Standard is the periplasmic (glycosylated form, external invertase) with 50% of its mass as polymannan. The periplasmic invertase molecule can exist in a number of association states each a multiple of the core glycosylated monomer, a 60 kDa peptide plus oligosaccharide chains and, depending on extraction, purification, and storage conditions, will exist as a dimer, tetramer, hexamer, or octamer. Since yeast can provide an alternative system for protein glycosylation that is similar to mammalian systems, periplasmic invertase is often used as a model for the study of the function of oligosaccharides in glycoproteins and for studies on glycoprotein biosynthesis.
R 1153 NEW	RNase B Glycoprotein Standard	Bovine pancreatic Ribonuclease B (RNase B) is a glycoprotein that contains only N-linked glycans. It is a globular protein composed of a single domain that occurs naturally as a lesser component in a mixture along with Ribonuclease A (RNase A) which is the non-glycosylated core form. RNase B contains a single glycosylation site at Asn ³⁴ at which from five to nine mannose residues are attached to the chitobiose core, i.e. Man ₅ GlcNAc ₂ , Man ₆ GlcNAc ₂ , Man ₇ GlcNAc ₂ , Man ₈ GlcNAc ₂ and Man ₉ GlcNAc ₂ . Due to the heterogeneity in the glycosylation at Asn ³⁴ , RNase B exists as five glycosylated variants, with a molecular weight of approximately 15,000 Da.

Key Glycoproteins for Scientific Research

G 9885	α₁-Acid Glycoprotein , human	99%, Purified from Cohn Fraction VI
A 5566	Monoclonal Anti-α₁-Acid Glycoprotein antibody	produced in mouse, Clone AGP-47, Ascites fluid, liquid
A 0534	Anti-α₁-Acid Glycoprotein antibody	produced in rabbit, IgG fraction of antiserum, Lyophilized powder
A 3418	Anti-α₁-Acid Glycoprotein antibody	produced in goat, Whole antiserum, liquid
A 9285	α₁-Antichymotrypsin from human plasma	approx. 95% (SDS-PAGE) Lyophilized powder Glycoprotein is specific inhibitor of chymotrypsin-like serine proteases. Contains Tris buffer salt and NaCl
A 9024	α₁-Antitrypsin from human plasma	Salt-free, lyophilized powder, α ₁ -Proteinase inhibitor, serine protease inhibitor; inhibits trypsin, chymotrypsin, pancreatic and granulocytic elastase, and acrosin. Effective concentration equimolar with proteinase. Chromatographically prepared and partially purified.
G 0516	α₂-hs-Glycoprotein from human plasma	minimum 90% (SDS-PAGE) Lyophilized from 20 mM Tris-HCl, pH 8.0, with 200 mM NaCl
A 1960	Aggrecan from bovine articular cartilage	Lyophilized powder, salt essentially free sterile-filtered (dialyzed against water)
A 2512	Albumin from chicken egg white (Ovalbumin)	Grade VI approx. 99% (agarose gel electrophoresis) Lyophilized powder Extent of labeling 5-6 mol mannose per mol ovalbumin protein
A 5503	Albumin from chicken egg white (Ovalbumin)	Grade V minimum 98% (agarose gel electrophoresis) Lyophilized powder salt essentially free
A 5378	Albumin from chicken egg white (Ovalbumin)	Grade III minimum 90% (agarose gel electrophoresis) Lyophilized powder
A 4781	Asialofetuin from fetal calf serum	Type I, salt essentially free N-acetylneuraminic acid <0.5%

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A 9791	Asialoglycophorin from human blood Type MN	Lyophilized powder, Predominantly Asialoglycophorin A
G 7900	Monoclonal Anti-Glycophorin A (a) antibody	Produced in mouse, Ascites fluid, Clone E4, Liquid
G 7650	Monoclonal Anti-Glycophorin A,B (a,d) antibody	Produced in mouse, Clone E3, Ascites fluid, Liquid
G 7775	Monoclonal Anti-Glycophorin C (b) antibody	Produced in mouse, Clone E5 or 2C10, Whole antiserum, Liquid
B 9277	Monoclonal Anti-Band 3 antibody	Produced in mouse, Clone BIII-136, Ascites fluid, Liquid
R 4011	Monoclonal Anti-Red Cell Wrb Antigen antibody	Produced in mouse, Affinity purified, One unit will bind 1.0 µg of d-biotin binds to Wrb, a composite blood group antigen resulting from the association between Glycophorin A and Band 3
A 8706	Avidin from egg white, recombinant	Affinity purified, One unit will bind 1.0 µg of d-biotin
B 8041	Biglycan from bovine articular cartilage	Essentially salt-free, lyophilized powder, sterile-filtered Interacts with collagen type I, as well as with fibronectin and TGF-β.
C 1063	Chorionic gonadotropin human	Lyophilized powder, sterile-filtered vial of 2,500 I.U.
C 5297	Chorionic gonadotropin human	Lyophilized powder, Potency: approx. 3,000 I.U. per mg
G 4877	Gonadotropin from pregnant mare serum	1,500-6,000 I.U./mg, PMSG
C 0755	Conalbumin chicken egg white (Ovotransferrin)	Substantially iron-free, Minimum 98%
D 8428	Decorin from bovine articular cartilage	Salt-free, lyophilized powder, sterile-filtered Decorin interacts with collagen type I and II, fibronectin, thrombospondin, and TGF-β.
F 2379	Fetuin from fetal calf serum	Lyophilized powder (from sodium acetate buffer) free <i>N</i> -acetylneuraminic acid approx. 0.2%
F 3004	Fetuin from fetal calf serum	Lyophilized powder Further processing of F 2379 by gel filtration.
F 3879	Fibrinogen from human plasma	Type I, Contains approx. 15% sodium citrate and approx. 25% sodium chloride. Approx. 60% protein (80-90% of protein clottable)
F 4385	Fibrinogen from murine plasma	Contains approx. 20% sodium citrate and approx. 30% sodium chloride. Approx. 50% protein (over 80% of protein clottable)
F 8630	Fibrinogen from bovine plasma	Type I-S powder, powder containing approx 75% protein, approx. 10% sodium citrate and approx. 15% sodium chloride
F 4883	Fibrinogen from human plasma	Essentially plasminogen-free powder containing approx. 50% protein, approx. 20% sodium citrate and approx. 30% sodium chloride
H 7017	Hemocyanin Megathura crenulata (keyhole limpet)	Lyophilized powder, contains stabilizing buffer, mol wt 8,000-9,000 kDa vial of 20 mg KLH (in approximately 100 mg total weight)
H 8283	Hemocyanin Megathura crenulata (keyhole limpet)	in PBS solution, Chromatographically purified Concentration 3-7 mg/ml protein (A ₂₈₀)
K 3009	Keratan sulfate proteoglycan from bovine cornea	sterile-filtered
L 0520	Lactoferrin from human milk	approx. 90% (SDS-PAGE) Lyophilized powder Chromatographically purified. Contains sodium chloride
L 2020	Laminin from Engelbreth-Holm-Swarm murine sarcoma	1 mg/ml in Tris buffered NaCl cell culture, tested sterile-filtered
M 1778	Mucin from porcine stomach	Type III partially purified powder, Bound sialic acids, approx. 1%
M 3895	Mucin from bovine submaxillary glands	Type I-S Lyophilized powder, Neuraminidase substrate Bound sialic acids approx. 12%

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Key Glycoproteins for Scientific Research

P 6782	Peroxidase horseradish	Essentially salt-free, Lyophilized powder 250-330 units/mg solid (using pyrogallol) Type VI-A
P 8375	Peroxidase horseradish	Type VI Essentially salt-free, lyophilized powder 250-330 units/mg solid
P 5661	Plasminogen from human plasma	ϵ -Aminocaproic acid free, Lyophilized powder containing NaCl, EDTA, lysine, and Tris buffer, plasmin <0.001 unit/unit plasminogen
T 1001	Thyroglobulin from bovine thyroid	Powder, Electrophoretically heterogeneous
T 1408	holo-Transferrin bovine	approx. 98%, iron-saturated
T 4132	holo-Transferrin human	minimum 98%, iron-saturated