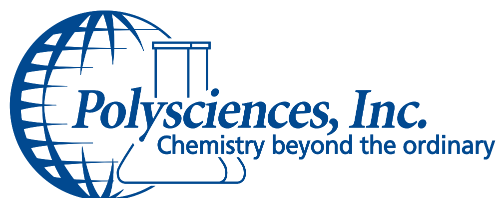

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TECHNICAL DATA SHEET 738

GlycoGel Stain Kit

Catalog #24693

INTRODUCTION

Glycoproteins are macromolecules composed of a protein and a carbohydrate or oligosaccharide. Additions of sugars can occur at asparagine sites and are called N-Glycosylation or at serine or threonine and are called O-Glycosylation. A Monosaccharide that is common in glycoproteins is glucose.

While these proteins are common in nature, they are relatively rare in bacteria. They occur in cells and show a phenomenon called “microheterogeneity” meaning that a variety of structurally different carbohydrate units can be found at even a single attachment site. Ovalbumin contains one glycosylated amino acid but over a dozen different oligosaccharides. Soluble glycoproteins show a high viscosity such as seen in egg whites.

Sugar groups assist in protein folding (usually found in the Endoplasmic reticulum) and improve the stability of the glycoprotein. Glycoproteins are essential to the immune system, especially in mammals. Some glycoproteins are present in blood group antigens, hormones (such as Thyroid Stimulating Hormone) and Erythropoietin (EPO) a major component to red cell production.

The absences of glycoproteins can lead to major diseases classified as “Glycogen Storage Diseases” which encompass over 40 diseases such as Mannosidosis, Mucolipidosis and Sialidosis. Specific glycoproteins have been identified in many diseases. Some mucin glycoproteins are associated with asthma and lung health, some with Cystic Fibrosis and others are linked to colon and bowel disorders. Glycoproteins are associated with muscular and neurodegenerative disorders, such as Alzheimer’s and Muscular Dystrophy.

Viruses, bacteria and parasites take advantage of cell surface carbohydrates associated with glycoproteins, using them to gain access to enter a cell. For example, rhinoviruses use I-Cam to gain entry, while parvovirus B19 uses an erythrocyte specific antigen (P antigen) to infect red cells. Helicobacter Pylori uses a Lewis blood group antigen on the surface of gastric mucinous cells to gain access.

Glycoproteins play an integral part in both our health and well being and in the discovery of disease states and how those diseases will be treated. Glycoproteins and their carbohydrate links are being investigated as drug delivery systems to treat and cure disease.

MATERIALS IN GLYCOGEL STAIN KIT

Cat. #	Description	Size
24693	GlycoGel Stain Kit (material for approx. 10 gels; 100 ml/gel)	1 kit

Contents of Kit (Ready to Use):

Schiff’s Reagent (Glycoprotein Stain)	1000 mL
0.5% Periodic Acid Aqueous (Oxidizing Solution)	1000 mL
0.55% Potassium Metabisulfite (Reducing Solution)	1000 mL

ADDITIONAL MATERIALS NEEDED

Hotplate and water bath
Vortex Genie
Orbital mixer
Micro centrifuge
Electrophoresis unit
5, 10, 20mL Pipets, 0-1000µl dispensing pipettes and tips
Graduated cylinder
Glass dishes
Tris-Glycine-SDS Buffer (Cat. #24091)
Methanol (Cat. #08032), dilute to 50% concentration
Acetic Acid, diluted to 3% solution in water
Electrophoresis Gels

FIX, STAIN AND DEVELOP GELS USING THE GLYCOGEL STAIN KIT REAGENTS

1. Prepare protein samples by transferring aliquots of each protein into 1.5ml microcentrifuge tubes containing 10µl of SDS-PAGE Reducing Solution, and 5 µl of Loading Dye. Vortex and boil samples for 5 min. in a water bath. After boiling, centrifuge samples for 10 seconds at maximum speed.
2. Prepare gels and Tris-Glycine-SDS Running Buffer. Load 25µl of each protein sample to each lane.
3. Run gels at 150 to 200 volts for 1.5 to 2 hrs

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PROCEDURE

1. Fix the gel in a bath of 100ml 50% Methanol (Cat. #08032) in deionized water. The gel will shrink in size as it is being fixed. The gel may be left in this solution overnight.
2. Wash the gel 2 times using two 100ml changes of deionized water. Discard the water in between washes. The gel will enlarge back to its original size.
3. Add 100ml of Oxidizing Solution, cover and mix on orbital mixer for a minimum of 1 hour. Pour off the stain into the proper waste stream for disposal.
4. Re-wash the gel using two 100ml deionized water washes and orbital mixing for 20 minutes, discard the water after each wash.
5. Stain the gel with 100ml of Glycoprotein Stain for a minimum of 1 hour or until the bands turn a magenta color.
6. Pour off the stain into the proper waste stream for disposal, and add 100ml of the Reducing Solution. Mix for 2 hours on the orbital mixer. Pour off the Reducing Solution into the proper waste stream for disposal.
7. Re-wash the gel using two 100ml deionized water washes and mix on the orbital mixer for 20 minutes. Pour off the water after each wash. The color bands will darken as will the gel itself.
8. Add 100ml of Storage Solution (5% acetic acid) and continue to mix. Mixing overnight is OK. Gradually the gel will lighten in color but the bands will remain magenta colored. Be careful not to wet the gel with water, or it will turn pink again.

STORAGE AND HANDLING

Store kit at 4° C.

ORDERING INFORMATION

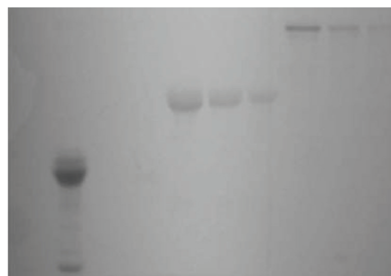
Cat. #	Description	Size
24693	GlycoGel Stain Kit	1 kit

ADDITIONAL PRODUCTS

Cat. #	Description	Size
08032	Methanol	6 x 1pt
24091	Tris-Glycine-SDS Buffer	5pk / 10pk
Acrylamide/Bis premixed powders		
17452	Acrylamide/Bis (19:1)	6 x 30g
19847	Acrylamide/Bis (29:1)	30g, 6 x 30g
17451	Acrylamide/Bis (37.5:1)	30g, 6 x 30g
PolyPAGE-40 Acrylamide/Bis liquid solutions (40% wt./vol.)		
24170	(19:1) liquid solution	100mL, 500mL, 6x100mL
24169	(29:1) liquid solution	100mL, 500mL, 6x100mL
24165	(37.5:1) liquid solution	100mL, 500mL, 6x100mL

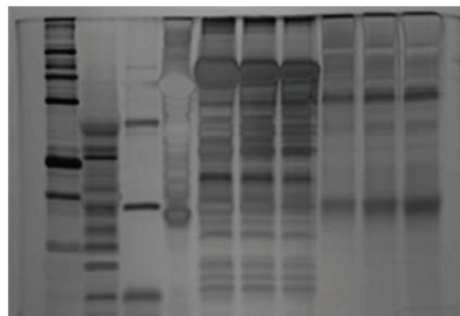
RESULTS

Figure 1
GlycoGel Stained Gel



Lane 1: NA, **Lane 2:** (+) CTRL Horseradish Peroxidase, **Lane 3:** NA, **Lane 4:** NA, **Lane 5:** 20µg Apo-Transferrin, **Lane 6:** 10µg Apo-Transferrin, **Lane 7:** 5µg Apo-Transferrin, **Lane 8:** 20µg Fibronectin, **Lane 9:** 10µg Fibronectin, **Lane 10:** 5µg Fibronectin

Figure 2
Silver Stained Gel



Note that the Silver Staining does not allow for "only glycoprotein" detection to visibly appear.

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Additional Glycoprotein Information for Positive Control Samples

APO-TRANSFERRIN (POSITIVE CONTROL)

SDS-PAGE shows a tight cluster of bands, corresponding to a molecular weight of approx 77,000 and a dimer may appear under certain conditions. The insolubility of Fe3+ at physiological pH requires that organisms transport this essential nutrient as a metal chelate. The transport agent in mammals is the serum protein transferrin. One aspect of transferrin chemistry is the kinetics of iron release to low molecular weight chelating agents. The mechanism for this reaction is very complex. There appears to be two pathways for iron release, and the relative importance of each pathway varies among different types of ligands.

The reaction rates are also strongly affected by the binding of inorganic anions to an allosteric regulatory site on the protein. This research is relevant to normal iron metabolism and to the search for an effective drug for treating iron toxicity.

In addition to its role in iron metabolism, transferrin also acts as the primary transport agent for a variety of toxic and therapeutic metal ions. One example is the evaluation of the role of transferrin in the serum transport of Al3+. Studies are being conducted on the binding of aluminum by serum chelating agents, on the exchange of aluminum between transferrin and low molecular weight ligands, and on computer models for the speciation of Aluminum in serum and cerebral spinal fluid.

Many of the current studies on transferrin involve the use of recombinant transferrin and single point mutations of transferrin. These studies involve collaborations with molecular biologists who produce specific mutants for chemical studies. These collaborative efforts now make it possible to evaluate the influence of specific amino acid side chains on metal binding affinities and the rates of metal exchange.

Now that there is a significant data base of binding affinities available, this research is focusing more on evaluating the specific molecular factors that govern metal binding affinity and selectivity. This includes the development of acid-base parameters for the protein and selected metal ions that will enable one to predict the transferrin binding constants for metals like Plutonium (Pu4+), which are extremely hazardous and thus difficult to study directly.

FIBRONECTIN (POSITIVE CONTROL)

Fibronectin is a high molecular weight glycoprotein containing about 5% carbohydrate that binds to receptor proteins called integrins which span the cell membrane. In addition to integrins, they also bind extra-cellular matrix components such as collagen, fibrin and heparin.
Fibronectin can be found in the blood plasma in its soluble form which is composed of two 250 kDa subunits joined together by disulfide bonds.

Plasma fibronectin is made in the liver by hepatocytes. The insoluble form that was formerly called cold-insoluble globulin is a large complex of cross-linked subunits.

There are several isoforms of fibronectin all of which are the product of a single gene. The structure of these isoforms are made of three types of repeated internal regions called I, II and III which exhibit different lengths and presence or absence of disulfide bonds. Alternative splicing of the Pre-mRNA leads to the combination of these three types of regions but also to a variable region. Fibronectin is involved in the wound healing process and so can be used as a therapeutic agent. It is also one of the few proteins whose production increases with age without any associated pathology.

HORSERADISH PEROXIDASE (HRP - POSITIVE CONTROL)

Horseradish peroxidase is isolated from horseradish roots (Amoracia rusticana) and belongs to the ferroprotoporphyrin group of peroxidases. HRP is a single chain polypeptide containing four disulfide bridges. It is a glycoprotein containing 18% carbohydrate. The carbohydrate composition consists of galactose, arabinose, xylose, fucose, mannose, mannosamine and galactosamine depending upon the specific isozyme. ***HRP's molecular weight (~44 kDa)*** includes the polypeptide chain (33,890 Daltons), hemin plus Ca2+ (~700 Daltons), and carbohydrate (~9,400 Daltons). At least seven isozymes of HRP exist. The isoelectric point for horseradish peroxidase isozymes ranges from 3.0 - 9.0.

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