

Product Information

GlycoProfile™ III Fluorescent Glycoprotein Detection Kit

Product Code **PP0300**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

Protein glycosylation is an important post-translational modification (PTM) commonly encountered in proteomic analysis. It is estimated that more than half of all proteins are glycoproteins. Identification of the glycoproteins within a sample plays a critical role in studying this PTM.^{1,2} Proteomic analysis often includes 1 or 2-dimensional electrophoresis of proteins, which is followed by a number of detection techniques.³

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 (Product Code GlycoPro). PAS staining of glycoproteins is very selective, but lacks the sensitivity of fluorescent detection (5–25 ng of carbohydrate).^{4,5}

Components

Sufficient reagents are supplied for 10 minigels.

Oxidation Reagent (Product Code O 2014) -
10x periodic acid solution in water.
One bottle containing 250 ml.

Glycoprotein Staining Reagent (Product Code G 8418)
100x dansyl hydrazide dye concentrate in acetonitrile.
One bottle containing 10 ml.

Staining Buffer (Product Code W 2014) - 50 mM sodium acetate, pH 4.75, with 500 mM NaCl, 10% ethanol, and 5% DMSO. It is used as the diluent for the staining reagent.

Two bottles containing 500 ml each.

ProteoProfile PTM Marker (Product Code P 1745) –
A buffered solution in 25% glycerol containing 4 proteins at a concentration of 1 mg/ml each. It is designed as both a positive and negative control for SDS-PAGE gels and Western blots of proteins with post-translation modifications. See Appendix I for additional information regarding the properties of the proteins in this marker
One vial containing 100 µl.

Equipment and Reagents Required But Not Provided.

- Acetic acid, Product Code A 6283
- Methanol, Product Code M 1775
- Ultrapure water, Product Code W 4502 or equivalent
- Orbital mixer or rocker table
- Minigel wash tray (100 ml capacity)
- SDS-PAGE sample loading buffer (Laemmli 2x Sample Buffer, Product Code S 3401)

Optional Reagents

- EZBlue™ Gel Staining Reagent, Product Code G 1041
- ProteoProfile™ Trypsin In-Gel Digest Kit, Product Code PP0100

Precautions and Disclaimer

This product is for laboratory research use only, not for drug, household, or other use. Consult the MSDS for information regarding hazards and safe handling practices. It is recommended to read the entire technical bulletin prior to starting the procedure.

Storage/Stability

It is recommended to store GlycoProfile III and all its components at 2-8 °C. The product as supplied is stable for one year when stored properly.

The Staining Buffer can be stored at 2-8 °C or at room temperature and is stable indefinitely.

After dilution in sample loading buffer, the ProteoProfile PTM Marker should be discarded after one day.

The Glycoprotein Staining Reagent should be used immediately after dilution and must be protected from light.

Procedure

This procedure is for staining a standard 10 x 10 cm SDS-PAGE minigel, but may be adapted for use with other sized gels. It is important to completely submerge the gel with constant mixing to ensure a uniform reaction across the gel. The volumes of reagents are relative to an appropriately sized wash/staining tray. After imaging of the fluorescent bands, counterstaining with a total protein stain (EZBlue Gel Staining Reagent) is strongly recommended to aid in the interpretation of the protein pattern. Staining with GlycoProfile III is compatible with in-gel trypsin digestion of the stained proteins and MALDI-MS analysis for protein identification.

Note: The fluorescent dye is subject to photobleaching. During steps 6-11 the gel and reagents should be protected from exposure to light. The stained gel must be protected from light to preserve the fluorescent bands.

1. Dilute the ProteoProfile PTM Marker in an appropriate sample loading buffer (not provided) prior to applying to the gel. Add 10 µl of ultrapure water to 5 µl of the ProteoProfile PTM Marker and then dilute this mixture 2-fold with 15 µl of Laemmli 2x Sample Buffer (Product Code S 3401). Incubate the solution in a boiling water bath for 3-5 minutes, or as appropriate for the loading buffer used. Then load 3 to 5 µl of the prepared marker onto the gel. This results in 500-800 ng of protein per band. Protein samples should be diluted and loaded to give protein content similar to the marker.
2. Perform electrophoresis using standard techniques.
3. Remove the gel from the cassette and transfer to a minigel wash/staining tray. Fix the proteins in the gel by adding 100 ml of a fixing solution (3% acetic acid with 50% methanol) and mixing for 60 minutes at 50-60 rpm on an orbital mixer or rocker table.
4. Discard the fixing solution and wash the gel 2 times with 100 ml of water. Each wash should be for 30 minutes.

Note: The gel can be stored in fixing solution or water overnight at 2-8 °C.
5. Dilute 10 ml of the Oxidation Reagent to 100 ml with water to make the Oxidation Solution. Mix well.
6. Discard the final wash water and incubate the gel in 100 ml of the Oxidation Solution for 20 minutes with constant gentle mixing. **Protect the gel from light during this oxidation step and through the remaining steps of the procedure.**

Note: If the gel has been stored at 2-8 °C, wash with room temperature water for 15 minutes prior to incubation of the gel in the Oxidation Solution.
7. Discard the Oxidation Solution and wash the gel with 100 ml of water for 5 minutes.
8. Dilute the Glycoprotein Staining Reagent with the Staining Buffer immediately before use. To stain a 10 x 10 cm minigel, dilute 1 ml of Glycoprotein Staining Reagent with 100 ml of Staining Buffer (Use a proportionate volume for larger gels). Mix well. **The Glycoprotein Staining Reagent should be diluted immediately before use and then protected from light.**
9. Submerge the gel in the prepared staining solution and cover. Stain the gel with constant gentle mixing for 60-90 minutes. Longer staining times will increase background fluorescence and may increase non-specific staining. **Cover the staining tray with foil to protect from light.**
10. Discard the staining solution and wash the gel 2 times with 100 ml of water. Each wash should be for 30 minutes. It is important that the gel be thoroughly washed by being fully submerged and moving freely through the water. **Continue to protect the gel from light during the washes.**

11. Remove the gel from the final water wash and view the gel with a standard fluorescent UV-transilluminator with emission at 312 nm. The glycosylated proteins will be visible as fluorescent yellow bands. Record the image with any standard imaging program. **Do not leave the gel on the UV-transilluminator for extended periods as significant photobleaching can occur within 10 minutes.**
12. If the background fluorescence is unacceptably high, as can happen with some pre-cast gels, repeat the water washes until the background is lower. **The gel can be washed in water overnight if protected from light.**
13. It is strongly recommended that the gel be stained for total protein with EZBlue Gel Staining Reagent following the fluorescent imaging.

Note: It is important to record the fluorescent image of the gel prior to EZBlue staining. EZBlue quenches the fluorescent signal.

GlycoProfile III can also be used on PVDF membranes. After gel electrophoresis, the proteins are transferred to a PVDF membrane using standard procedures. The glycoproteins are then detected by following steps 5-11 of the staining procedure for gels.

Results

The limit of detection varies with the glycoprotein and the degree and type of glycosylation. Typical detection limits observed with the ProteoProfile PTM Marker 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.

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.

Fluorescent Band	Total Protein Band	Interpretation
Bright	Faint	Specific
Bright	Dark	Probably Specific
Faint	Faint	Possibly Specific
Faint	Dark	Non-specific

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.

References

1. Brush, M., Sugar and Spice: Glycobiology: The Next Frontier. *The Scientist*, **13**, 22 (1999).
2. Kuster, B., et al., Glycosylation analysis of gel-separated proteins. *Proteomics*, **1**, 350-361 (2001).
3. Packer, N. H., et al., Proteome Analysis Protocols. *Methods in Molecular Biology*, Volume **112**, 2-D, pp. 341-353.
4. Jay, G. D., et al., Silver staining of extensively glycosylated proteins on sodium dodecyl sulfate-polyacrylamide gels: enhancement by carbohydrate-binding dyes. *Anal. Biochem.*, **185**, 324-330 (1990).
5. Zacharius, R.M., et al., Glycoprotein staining following electrophoresis on acrylamide gels. *Anal. Biochem.*, **30**, 148-152 (1969).

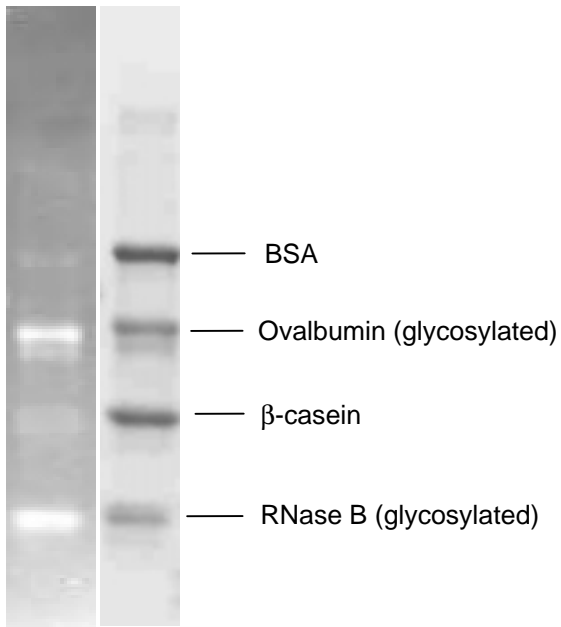
Figure 1

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.

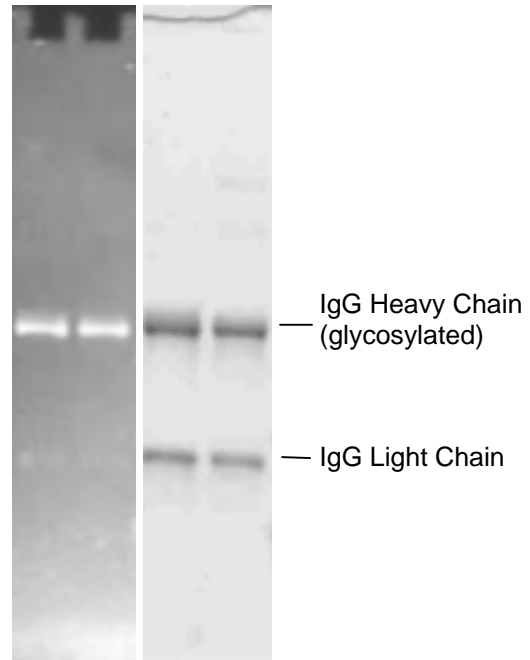
Figure 2

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.

Troubleshooting Guide

Problem	Cause	Solution
Background fluorescence is high.	Ineffective washing due to poor mixing or improper tray size.	Choose a staining tray size that allows the gel to be completely submerged and gives sufficient movement of solutions around the gel.
	Gel was allowed to stain too long.	Optimal staining will be achieved in 60-90 minutes. Longer staining requires longer washing. Washing times may be extended for several hours to overnight if protected from light. The gel can be wrapped in plastic and stored overnight for rewashing the next day if desired.
	SDS, buffers, or salts from electrophoresis were not thoroughly washed from gel.	Follow recommendations for fixing and washing the gel prior to oxidation.
	Differences in gel manufacture.	Gels manufactured by different companies absorb the dye to varying degrees and some require additional water washes. Wash with 100 ml of water for 30 minutes and image again. Repeat this additional wash until the background is acceptable. This is especially helpful with long shelf-life gels.
Fluorescent blotches on gel	Gel may have become stuck to staining tray.	Ensure the gel is moving freely in the solutions during all staining and washing steps.
	Staining tray was not clean.	Clean the tray with soap and water, rinsing with ultrapure water, prior to staining.
	Staining solution was not homogenous.	After the Glycoprotein Staining Reagent is diluted with the Staining Buffer, mix well before adding to the gel.
Low glycoprotein sensitivity	Low level of glycosylation.	Detection is determined by the amount of carbohydrate, not simply protein content. It may be necessary to load more protein onto the gel.
	Carbohydrate moieties with low reactivity towards the dye or periodate oxidation.	The type of glycosylation will affect sensitivity. Two proteins of identical sugar content may be detected differently if the sugars are of dissimilar composition or if there are steric interferences.
Loss of sensitivity in total protein staining with EZBlue Gel Staining Reagent	The reaction of the fluorescent dye with the glycoprotein can block the EZBlue Gel Staining Reagent reactivity.	If the total protein profile in a sample needs to be determined, it is best to run two identical gels, staining one for glycoproteins and the other for total proteins.
Appearance of a very low molecular weight band (<7 kDa) with very high fluorescence	Electrophoretic artifact possibly associated with the tracking dye or SDS micelles.	Rinsing the gel with water for 15 to 20 minutes prior to fixing can help eliminate this band.

Appendix I

ProteoProfile™ PTM MARKER

Post Translational Modification Marker

Product Number **P 1745**

Storage Temperature 2-8 °C

Product Description

Glycosylation and phosphorylation are the protein post-translational modifications (PTM) most frequently encountered in proteomic analysis. The ProteoProfile™ PTM Marker contains glycosylated and phosphorylated proteins and is designed as both a positive and negative control for SDS-PAGE gels and Western blots of proteins with post-translation modifications.

Component

The ProteoProfile PTM Marker is supplied as a solution of four proteins, each at 1 mg/ml, in 250 mM Tris buffer, pH 7, with 25% glycerol. Table 1 shows the phosphate and carbohydrate content of 1 µl of the marker. Properties of the four proteins contained in the marker are shown in Table 2.

Table 1.

Carbohydrate and phosphate content of 1 µl of the ProteoProfile PTM Marker

Protein	Carbohydrate (ng)	Phosphate (pmole)
Albumin	none	none
Ovalbumin	35	45
β-Casein	none	160
RNase B	200	none

Table 2.

Properties of Proteins in the ProteoProfile PTM Marker

Protein	MW (kDa)	Glycosylation	Phosphorylation
Albumin, bovine serum	66	none	none
Ovalbumin, chicken egg	45	1 N-linked glycan 3.2% carbohydrate ¹	2 phosphorylation sites (serine)* 0.3-0.4% phosphate by weight ¹
β-Casein, bovine milk	30	none	5 phosphorylation sites ² (serine)* 1.3-1.7% phosphate by weight
RNase B, bovine pancreas	17	2 N-linked glycans 19.5% carbohydrate ³	none

*Potential phosphorylation sites. Not all sites will be phosphorylated.

Storage/Stability

It is recommended to store the product at 2-8 °C. The product as supplied is stable for one year when stored properly. After dilution with sample loading buffer, the marker should be used within 24 hours.

Preparation Instructions

The marker must be diluted with an appropriate sample loading buffer, such as Laemmli 2x Sample Buffer (Product Code S 3401), prior to SDS-PAGE. The dilution and loading volume of the marker is determined by the dynamic range of the detection method and the marker concentration should be similar to that of the sample.

For fluorescent detection of glycoproteins (detection limit of 5–25 ng of carbohydrate) on SDS-PAGE gels or Western blots (GlycoProfile III, Product code PP0300), 3-5 µl of a 6-fold dilution of the marker should be loaded.

References

1. [http://www.food-allergens.de/symposium-vol1\(1\)/data/egg-white/gald2.htm](http://www.food-allergens.de/symposium-vol1(1)/data/egg-white/gald2.htm)
2. Posewitz, M., and Tempst, P., *Anal. Chem.*, **71**, 2883-2892 (1999).
3. Plummer, T., and Hirs, C., *J. Biol. Chem.*, **238**, 1396 (1963).

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