

Rapid Fractionation and Enrichment of Glycoproteins for Analysis by 2D Electrophoresis

Glycoproteins are proteins that are post-translationally modified by the addition of carbohydrates. The carbohydrates are coupled to asparagine (N-linked) and serine/ threonine (O-linked) residues during passage through the endoplasmic reticulum and golgi apparatus. They are commonly found decorating the cell membrane with the carbohydrate moieties in the extracellular space. Glycosylated proteins play critical roles in cell signaling, inflammation, cell-to-cell adhesion and in the immune response.

FOCUS™ Glycoprotein rapidly fractionates glycoproteins that have terminal α -D-mannosyl and α -D-glycosyl residues. FOCUS™ Glycoprotein utilizes spin columns containing the immobilized lectin Concanavalin A for rapid glycoprotein isolation.

AIM

To evaluate FOCUS™ Glycoprotein kit in the fractionation of Jurkat cells. After fractionation, fractions were analyzed by 1D electrophoresis stained with Reversible Zinc Stain™ and 2D electrophoresis stained with a fluorescent protein stain.

METHOD

Jurkat cells (20×10^6) were harvested and washed in phosphate buffered saline (PBS) and centrifuged at 500g for 2 minutes to pellet the whole cells. 0.3ml Glyco-Loading Buffer, supplemented with 3 μ l 100X ProteaseArrest™ protease inhibitor cocktail, was added and the cells were sonicated on ice for 2 minutes in 30 second bursts. The cell debris was removed by centrifugation at 16,000g for 10 minutes at 4 °C.

The Glyco-Column was first equilibrated with 2ml Glyco-Loading Buffer by adding 0.4ml batches Glyco-Loading Buffer and centrifuging the spin columns at 500g for 10 seconds. The Jurkat cell supernatant was applied to the column, which was incubated at room temperature for 30 minutes. The column was washed five times with a total volume of 2ml Glyco-Loading Buffer. For elution of the glycoproteins, 2 x 0.2ml Glyco-Elution Buffer I was added and the column was incubated for 15 minutes. The column was centrifuged at 500g for 10 seconds and the eluent collected. The elution step was repeated with 0.2ml Glyco-Elution Buffer II and then Glyco-Elution Buffer III and the fractions were collected separately.

10 μ l Crude Lysate (CL), the flow through (FT), the

five washes (W1-5) and the four eluents (E1-4) were mixed with SDS-PAGE Sample Loading Buffer, boiled and loaded onto a 4-20% SDS-PAGE polyacrylamide gel. The proteins were resolved and visualized with Reversible Zinc Stain™.

60 μ l of eluents 1, 2 and 3 were combined and treated with Perfect-FOCUS™ to remove any 2D electrophoresis contaminating agents. 20 μ l crude lysate was also treated. Following Perfect-FOCUS™ treatment, the samples were loaded on to 11cm pH3-10 isoelectric focusing strips, followed by 4-20% SDS-PAGE. The proteins were detected with a fluorescent protein stain.

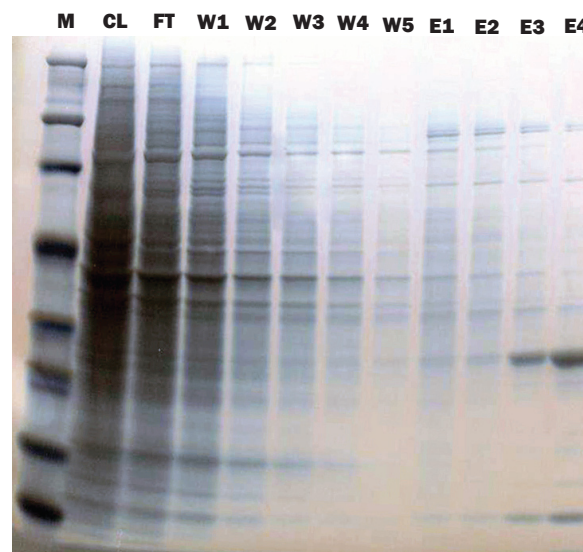


Figure1: FOCUS™ Glycoprotein isolates a large number of glycoproteins. Jurkat cells were lysed by sonication, centrifuged and the supernatant (CL) loaded onto a FOCUS™ Glycoprotein column. Following incubation the column was centrifuged and the flow through (FT) was collected. The column was washed 5 times (W1-5) and the glycoproteins were sequentially eluted with Glyco Elution Buffer I (E1-2), Glyco Elution Buffer II (E3) and Glyco Elution Buffer III (E3).

10 μ l was loaded onto a 4-20% SDS-PAGE gel and the proteins were resolved and visualized with Reversible Zinc Stain™. The gel was placed on a black background and scanned.

RESULTS AND DISCUSSION

Figure 1 shows the protein profile of Jurkat cells treated with FOCUS™ Glycoprotein. As expected, the majority of the Jurkat cell proteome is removed in the flow through and washing steps as only a small percentage of the proteome is glycosylated. A large

number of glycosylated proteins are isolated from the FOCUS™ Glycoprotein columns and the protein profiles change when each Glyco-Elution Buffer is used.

In addition, the low abundant proteins are now easily visualized in the elution fractions as the majority of the proteome has been removed and due to the high sensitivity of the Reversible Zinc Stain™.

Figure 2 is a comparison of the crude Jurkat cell lysate and an equal mix of the elutions E1, E2 and E3 from figure 1. Firstly, the use of the FOCUS™ Glycoprotein kit significantly reduces the complexity of the 2D map, making it easy to identify and isolate glycoprotein protein spots. Secondly, the concentration of the proteins is stronger due to the enrichment of the glycoproteins by the FOCUS™ Glycoprotein kit.

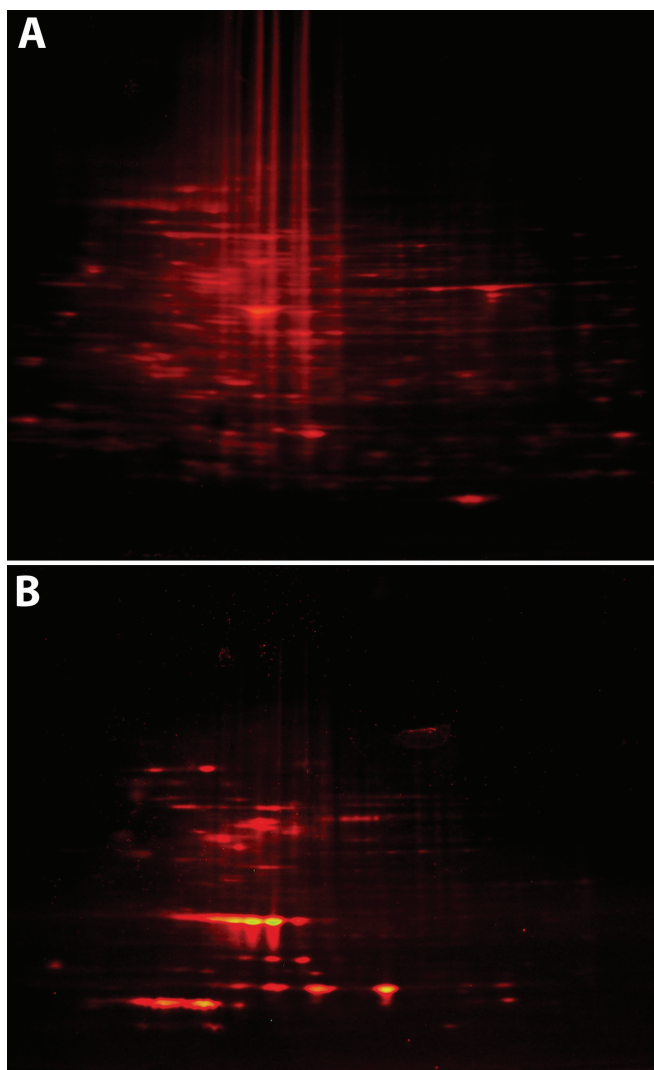


Figure 2: Comparison of crude Jurkat cell lysate (A) and glycoproteins (B) isolated with FOCUS™ Glycoprotein. Glycoproteins were isolated from Jurkat cells using FOCUS™ Glycoprotein kit as described in the Methods section. The first 3 elution fractions were combined and the eluents and a sample of the crude Jurkat cell lysate were treated with Perfect-FOCUS™ to prepare them for 2D electrophoresis. The first dimension was run on 11cm pH3-10 strips and the second dimension on 4-20% SDS polyacrylamide gels. Proteins were visualized with a fluorescent protein stain.

FOCUS™ Glycoprotein is ideal for the fractionation, enrichment and isolation of glycoproteins from a wide selection of samples, including tissues, cell lysates and serum. The fast and convenient spin column format bind and immobilize ~5mg glycoproteins and the enriched, eluted glycoproteins are ready for further analysis within 90 minutes.

ORDERING INFORMATION

VWR Cat. No.	Description	Size
82022-684	FOCUS™ Glycoprotein	10 Preps
82021-408	Reversible Zinc Stain™	25 mini gels
82022-478	ProteaseArrest™ [100X]	2 mL
82021-274	Perfect-FOCUS™	50 Preps
95029-160	Perfect-FOCUS™	6 Preps

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Perfect-FOCUS™

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