



FOCUS™ -Glycoprotein

INTRODUCTION

Fractionation and enrichment simplifies a complex protein mixture by reducing the amount and the number of protein species to be loaded into the gel matrix for iso-electric focusing (IEF) or 2D gel electrophoresis. Fractionation produces less crowded protein maps, simplifying analysis and interpretation. Separation or enrichment of glyco-proteins is one of the methods of reducing the complexity of a protein extract or serum sample.

FOCUS™ -Glycoprotein is an optimized method for enrichment and removal of glyco-proteins from complex protein samples. FOCUS™ -Glycoprotein kit is based on lectin binding for specific glycoproteins with terminal α -D mannosyl and α -D glycosyl proteins. The kit is provided in spin column format containing lectin (Concanavilin A) bond resin with the capacity to bind and immobilize ~5mg glyco-proteins. Column bound glyco-proteins are eluted with a set of three rapid elution buffers.

The kit is suitable for fractionation or enrichment of 10 samples (1-1.5mg protein each preparation) for IEF, 2D gel electrophoresis and other applications.

ITEMS SUPPLIED: Cat. # 786-253

Glyco-Loading Buffer	50ml
Glyco-Elution Buffer-I	5 ml
Glyco-Elution Buffer-II	5 ml
Glyco-Elution Buffer-III	5 ml
Glyco-Columns	10

STORAGE CONDITION:

Store refrigerated. When stored and used properly this kit is good for twelve months.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT:

Centrifuge and 1.5-2ml collection tubes.

PROTOCOLS

Perform the entire purification procedure at room temperature. Before use, allow the buffers to warm to room temperature.

Optional: If the inhibition of protease activity is required, add a cocktail of protease inhibitors to the buffers to prevent protease activities (see Related Products for protease inhibitor *ProteaseArrest*™, Cat. # 786-108).

Read the notes on **NOTE FOR CENTRIFUGATION** below before using the *Glyco-Column*™.

1. Equilibrate *Glyco-Column*™ :

Centrifuge the *Glyco-Column*™ briefly (5-10 seconds) at 200xg. Break open the bottom plug.

Place the *Glyco-Column*™ in a 2ml collection tube and centrifuge 5-10 seconds at 200xg. Apply 0.3-0.4ml *Glyco-Loading* Buffer to the column and centrifuge 5-10 seconds at 200xg. Repeat this wash step 4-5 times.

Centrifuge the column (5-10 seconds at 200xg) to remove the void volume of *Glyco-Loading* Buffer.

2. Protein sample preparation:

IMPORTANT NOTE: for optimal results the protein sample must have salt concentration 50-200mM (e.g., sodium, potassium, calcium salts, etc.).

Check the composition of protein-buffer. If the protein buffer does not contain sufficient salt concentration, mix with equal volume of *Glyco-Loading* Buffer to increase the salt concentration. If the salt concentration in protein solution is higher than 200mM, dilute the protein solution with pure water to reduce the salt concentration to around 50-200mM.



For best results, dilute the protein sample in *Glyco*-Loading Buffer or dialyze the protein sample against *Glyco*-Loading Buffer.

3. Load the protein sample on *Glyco*-Column™:

Each column is suitable for loading 1-2mg protein sample.

Replace the *Glyco*-Column™ in a clean collection tube. Apply (~400 µl) protein solution (about 1-1.5mg protein) on the column. Incubate the column for ~30 minutes. Reapply, every 5-10 minutes, any flow-through collected in the collection tube. If the protein sample does not enter the column, centrifuge the column for 5-10 seconds at 200xg and reapply the flow-through.

After 30 minutes incubation, centrifuge the column for 5-10 seconds at 200xg.

Collect and save the eluent until a satisfactory result is obtained. The eluent contains protein free from protein species containing terminal α -D mannosyl and α -D glycosyl.

4. Wash Steps.

Replace the *Glyco*-Column™ into a clean collection tube.

Wash the column 4-5 times with *Glyco*-Loading Buffer, ~400 µl each wash. Apply ~400 µl *Glyco*-Loading Buffer to the column and centrifuge 5-10 seconds at 200xg. Repeat the wash step 4-5 times.

5. Elution of glyco-proteins

For the maximum recovery of the glycoprotein captured by the *Glyco*-column™, use the following three-step elution.

Elution-I: Place the column in a clean collection tube and apply 0.2ml Glyco-Elution Buffer-I. Incubate the column for 15 minutes. Centrifuge the column for 5-10 seconds at 200xg.

Repeat above elution once with 0.2ml Glyco-Elution Buffer-I.

Elution-II: Apply 0.2ml Glyco-Elution Buffer-II. Incubate the column for 15 minutes. Centrifuge the column for 5-10 seconds at 200xg.

Elution-III: Apply 0.2ml Glyco-Elution Buffer-III. Incubate the column for 15 minutes. Centrifuge the column for 5-10 seconds at 200xg.

NOTE- The Eluents from the three elution steps may be combined in one tube. The eluents contain a salt concentration that may not be suitable for 2D gel analysis. Dialyze the eluent against an appropriate buffer. Alternatively, prepare and clean the sample with Perfect-FOCUS (Cat.# 786-124) before 2D gel analysis.

RE-USING *Glyco*-Column: Column may be regenerated and used one more time. For regeneration, apply 0.4ml Elution Buffer-III. Centrifuge the column for 5-10 seconds at 200xg. Repeat the wash step 4-5 times. Wash the column with loading buffer 4-5 times. Store the column in the loading buffer in the cold. Before use, equilibrate the column. Additional buffers may be purchased separately. Please note that if the column is not stored and used properly, the binding capacity of the column will deteriorate with time.

NOTE FOR CENTRIFUGATION -Centrifugation should not be too severe as to dry the column. Centrifugation should be at such a moderate speed that it removes only 70-80% of the buffer from the column, leaving behind in the column 20-30% buffer. If necessary, make a trial run (before loading the protein sample) to determine an appropriate centrifugation condition. Make note of the centrifugation speed and duration. Use the identical centrifugation condition at each step.

RELATED PRODUCTS

Perfect-FOCUS (Cat # 786-124): A kit for preparing sample for 2D gels.

FOCUS Nuclease™ (Cat. 786-039F): for removing nucleic acid contamination from samples.

FOCUS™-Soluble & Insoluble Protein (Cat. # 786-247): for fractionation of soluble and insoluble proteins.

FOCUS™ Membrane Protein (Cat. #786-249): for fractionation and enrichment of membrane proteins.

NOTE: For other related products, please visit our web site www.GBiosciences.com or contact us.

LE 11.04.08-SA/MM/IA