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A Geno Technology, Inc. (USA) brand name

FOCUS™ Glycoprotein

(Cat. # 786-253)

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 isoelectric focusing (IEF) or 2D gel electrophoresis. Fractionation produces less crowded protein maps, simplifying analysis and interpretation. Separation or enrichment of glycoproteins 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 glycoproteins 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 Concanavalin A (Con A) Agarose, which consists of Con A coupled to 6% agarose beads. Concanavalin A (Con A) Agarose has the capacity to bind and immobilize 15-30mg glycoproteins. Column bound glycoproteins are eluted with a set of three rapid elution buffers.

ITEM(S) SUPPLIED (Cat. # 786-253)

Description	Size
Glyco-Loading Buffer	50ml
Glyco-Elution Buffer-I	5ml
Glyco-Elution Buffer-II	5ml
Glyco-Elution Buffer-III	5ml
Concanavalin A (Con A) Agarose Columns	10 columns

STORAGE CONDITION

The kit is shipped at ambient temperature. Store refrigerated. When stored and used properly this kit is good for twelve months.

ADDITIONAL ITEMS REQUIRED

Centrifuge and 1.5-2ml collection tubes

RESIN SPECIFICATIONS

- Ligand Density: 10-16mg Con A/ml resin
- Capacity: 20-50mg thyroglobulin/ml resin
- Bead structure: 6% agarose
- pH Stability: 4-9

IMPORTANT INFORMATION

- Concanavalin A (Con A) Agarose requires Mn^{2+} and Ca^{2+} ions for carbohydrate binding, so buffers should either include these metal ions or the resin should be equilibrated with these ions immediately prior to binding.
- Avoid buffers with chelating agents (EDTA) as these will remove the essential Mn^{2+} and Ca^{2+} ions.

PROTOCOL

1. Perform the entire purification procedure at room temperature. Before use, allow the buffers to warm to room temperature.
   NOTE: *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).*
2. Equilibrate Con A Agarose Column: Centrifuge the Con A Agarose Column for 5-10 seconds at 200xg. Break open the bottom plug.
3. Place the Con A Agarose Column in a 2ml collection tube and centrifuge 5-10 seconds at 200xg. Discard the storage buffer.
4. Apply 0.3-0.4ml Glyco-Loading Buffer to the column and centrifuge 5-10 seconds at 200xg. Discard the wash buffer. Repeat this wash step 5 times.
5. Centrifuge the column for 5-10 seconds at 200xg to remove the void volume of Glyco-Loading Buffer.
6. Protein sample preparation: For optimal results the protein sample must have salt concentration 50-500mM and contain Mn^{2+} and Ca^{2+} ions. Check the composition of the 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 500mM, dilute the protein solution with pure water to reduce the salt concentration to around 50-500mM.For best results, dilute the protein sample in Glyco-Loading Buffer or dialyze the protein sample against Glyco-Loading Buffer.
7. Each column is suitable for loading 1-15mg protein sample.
8. Place the Con A Agarose Column in a clean collection tube. Apply 400µl protein solution to the column.
9. Incubate the column for ~30 minutes at room temperature. 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.
10. After 30 minutes incubation, centrifuge the column for 5-10 seconds at 200xg.
11. Collect and save the eluent until a satisfactory result is obtained. The eluent contains protein free from protein species containing terminal □-D mannosyl and □-D glycosyl.
12. Replace the Con A Agarose Column into a clean collection tube.

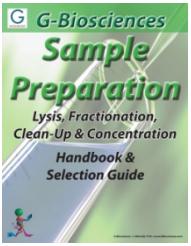
13. Wash the column 4-5 times with Glyco-Loading Buffer. Apply 400 µl Glyco-Loading Buffer to the column and centrifuge 5-10 seconds at 200xg. Repeat the wash step 4-5 times.
14. For the maximum recovery of the glycoprotein captured by the Con A Agarose Column, use the following three-step elution.
15. 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.
16. Elution-II: Apply 0.2ml Glyco-Elution Buffer-II. Incubate the column for 15 minutes. Centrifuge the column for 5-10 seconds at 200xg.
17. 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.*

CON A AGAROSE REGENERATION

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.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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Last saved: 8/28/2012 CMH

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