

Reduction in Sample Complexity Through Glycoprotein Enrichment

Proteomic Application Note

INTRODUCTION

Characterization of a complex protein mixture by 2DE requires the ability to resolve and identify proteins over a broad dynamic range. In order to resolve less abundant proteins, it is often necessary to reduce the complexity of a total protein mixture. It is common to employ any number of fractionation schemes to this end depending upon the user's objectives. These methods generally include fractionation based on size exclusion, charge, hydrophobicity, binding affinity, or some manner of enrichment. One such enrichment is based on lectin binding to specific glycoproteins. Concanavilin A (ConA) is a 55 kd protein isolated from *Canavalia ensiformis* (Jack Bean) and has an affinity for terminal α -D mannosyl and α -D glucosyl proteins. ConA can selectively enrich for glycoproteins with these specific moieties and reduce the complexity of a protein lysate or serum sample.

The Genomic Solutions Glycoprotein Enrichment Kit (part number 80-0235) contains concanavilin A bound to fast flow sepharose 4B in a spin column format. The kit is designed to be used upstream of isoelectric focusing and 2D electrophoresis. Protocols provided with the kit are intended as initial starting parameters only and these should be optimized according to the researcher's particular requirements.

PROTOCOLS

The protein sample to be applied to the ConA resin should be prepared so that the sample is in a buffer at physiological pH (6.8-7.0) since glycoprotein binding to lectins is pH dependent. That is, below pH 5.6 Con A exists as a dimer while above pH 7.0 higher order aggregates are formed. Additional precautions should be noted:

- 1) Dialysis of the sample will remove free sugars present in the cytoplasm or culture medium which may compete for binding sites.
- 2) Glycoprotein binding to ConA is inefficient in the presence of detergents. Also, disruption of cells with detergent results in the release of lysosomal proteases which might rapidly degrade or modify many membrane bound glycoproteins. Therefore, solubilization should be accomplished through the use of strong denaturants (urea or guanidine hydrochloride), chaotrophs (thiocyanate or iodide), or organic solvents (ethanol or isopropanol). Certain non-ionic detergents may be used for Con A, such as 0.1% NP-40, but will reduce the binding efficiency to 88%¹.

1. Harris, E.L., Angal, S. Protein Purification Methods: A Practical Approach. P277-281. Oxford University Press, 1995.

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Glycoprotein Enrichment Kit Protocol

1. Spin Columns for 2 minutes at 2000 rpm to remove storage buffer.
2. Add 400 μ L of Binding Buffer to column.
3. Spin for 5 minutes at 2000 rpm.
4. Discard Flowthrough.
5. Dilute sample to 400 μ L with Binding Buffer and add to column directly. Add sample (recommended < 1 mg total protein)
6. Rotate for 2 hours at room temperature.
7. Centrifuge for 6 minutes @ 2000 rpm and collect flowthrough.
8. Add 400 μ L of Binding Buffer to matrix and spin for 6 minutes @2000 rpm.
9. Repeat Step 8.
10. Wash in 400 μ L Wash Buffer (Eq buffer + 1.0% Triton X-100).
11. Add 350 μ L of Elution Buffer and rotate for 1 hour - overnight.
12. Collect eluant by centrifugation @ 4000 rpm x 5 minutes.
13. Add 7.5 μ L of 0.25N* NaOH directly to eluant and transfer to a Microcon™ Amicon YM-3 spin column. Centrifuge @14000 rpm x 60 minutes or until sample is concentrated to approx. 50 μ L. Invert column into a clean microcentrifuge tube and spin at 14000 rpm for 1 minute to collect concentrated eluant from the column. **Final pH of solution should be between 6.5-8.0 prior to concentration and rehydration of sample. This will vary depending upon the sample. Users may wish to check pH of a neutralized "dummy" sample to ensure proper pH neutralization prior to loading.*
14. Add rehydration buffer (Genomic Solutions part number 70-4019) to 350-400 μ L total volume.
15. Incubate at room temperature for 1 hour.
16. Centrifuge sample for 10 minutes to remove any precipitated salts.
17. Pipette supernatant to a clean tube.
18. Rehydrate IPG strips with entire volume overnight and focus according to manufacturer specifications.
19. 2DE gels shown were focused on the IPG pHaser™ system (Genomic Solutions part number 70-3537) overnight. Strips were equilibrated in Equilibration Buffers I and II (Genomic Solutions part number 70-3984 & 70-3982, respectively) for 10 minutes each and electrophoresed through a large format 10% precast Duracryl™ gel in the Genomic Solutions 2D Investigator™ System. (Genomic Solutions part number 80-0009).
20. Gel plugs for identification were excised using Genomic Solutions Investigator HT PC Analyzer software on a Genomic Solutions Investigator ProPic™ Robotic Workstation.

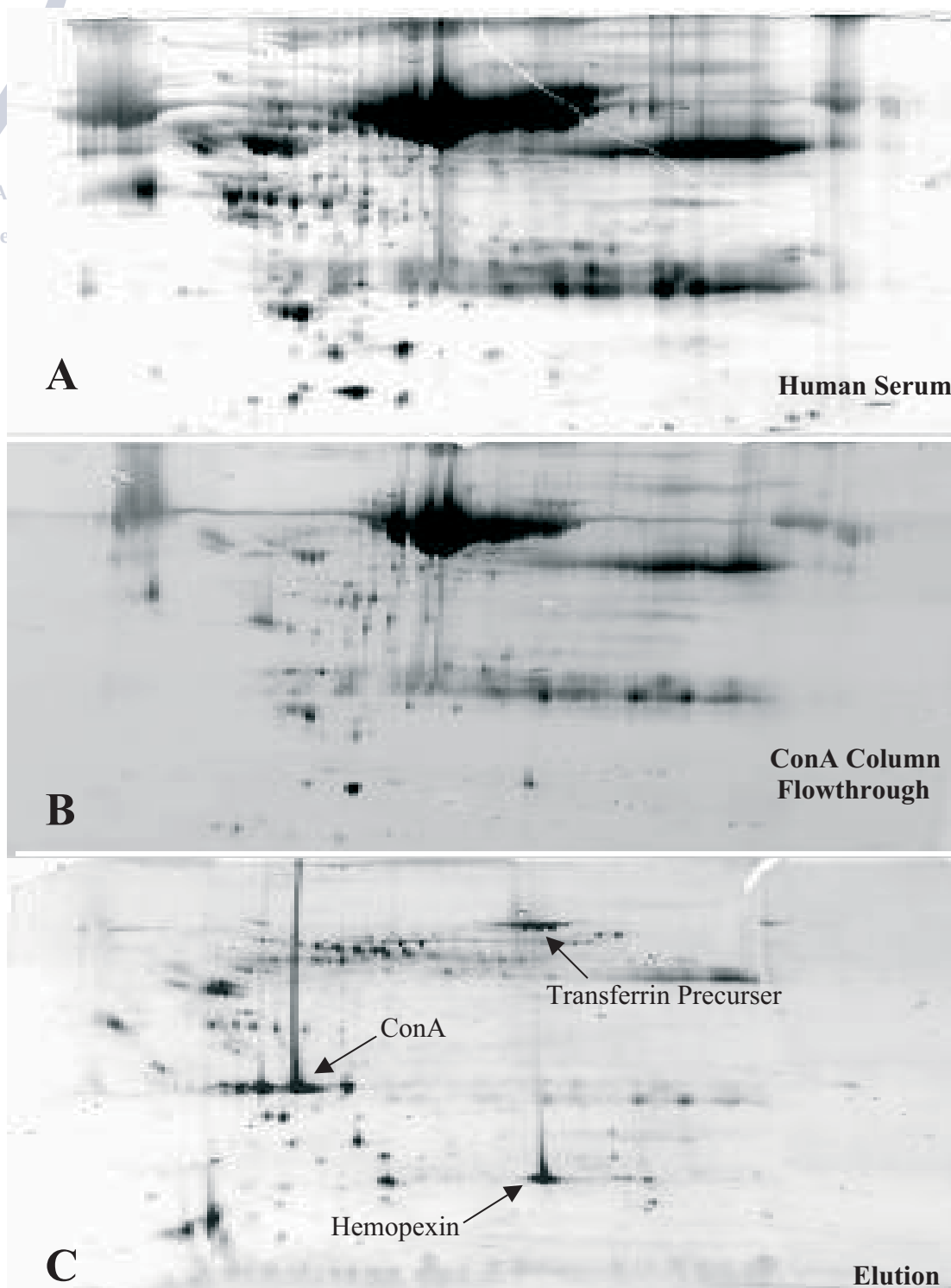
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Figure 1: 140 μ g of pooled human serum (Accurate[®] Chemical and Scientific Company) was subjected to the protocol in the text. 140 μ g of human serum is illustrated in A, the flowthrough fraction is illustrated in B and the column eluant is illustrated in C. The gels were stained with SYPRO[®] ruby. Protein identifications were performed on excised gel plugs by peptide mass fingerprinting in the Genomic Solutions Proteomic Facility using an Applied Biosystems Voyager-DETM STR MALDI TOF Mass Spectrometer.

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Glycoprotein Enrichment Kit: Part Number 80-0235

Kit Components:

Binding Buffer, 10ml

Wash Buffer, 3 ml

0.25N NaOH, 100 μ L

Elution Buffer, 2 ml

ConA Columns (6), each column contains 0.5-0.8 mg of packed ConA.

Amicon YM3 Columns (6)

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