MultiScreen® Filter Plate with Ultracel®-10 membrane: Concentration of proteins in cell lysate using a 10,000 nmwl ultrafiltration membrane in a 96-well plate format

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Introduction

A method for high throughput sample preparation has been developed using the MultiScreen filter plate with Ultracel-10 membrane (MultiScreen Ultracel-10 filter plate). The ultrafiltration-based filter plate is designed for automation-compatible sample purification, concentration and desalting of biological solutions. The 96-well MultiScreen filter plate incorporates Ultracel-10 ultrafiltration membrane (10,000 nominal molecular weight limit regenerated cellulose) for ultra low-binding, high recovery results. It is designed for use with centrifugation and is compatible with standard microtiter plates, instrumentation and liquid handling equipment.

This study demonstrates the use of the MultiScreen Ultracel-10 filter plate for concentration of whole cell lysates, without loss of protein and with high reproducibility across the plate. Applications include parallel protein purification, protein concentration and buffer exchange in cell lysates for subsequent separation or assay.

Part 1. Cell lysate protein concentration: ultrafiltration time, retentate volumes and concentration factors

Objective

The goals of these experiments were to determine the optimal time required to concentrate liver extract, to evaluate well-to-well reproducibility of the retentate volume and to obtain protein concentration factors using the MultiScreen Ultracel-10 filter plate. In each of the experiments, results using vacuum-driven filtration were compared to those using centrifugation-driven filtration.

Materials

Reagents

- Milli-Q® water
- Lysis Buffer (50 mM Tris pH 7.5, 150 mM NaCl)
- Protease Inhibitor Cocktail cat. P8340 (Sigma-Aldrich − St. Louis, MO)
- 50 mM Tris buffer pH 7.5
- BCA Protein Assay Kit cat. 23225 (Pierce Biotechnology − Rockford, IL)
- Albumin standard cat. 23209 (Pierce Biotechnology − Rockford, IL)
- Avian liver tissue

Equipment and materials

- MultiScreen filter plate with Ultracel-10 membrane cat. MAUF01010 (Millipore Corp. − Billerica, MA)
- 96-well polypropylene, V-bottom collection plate cat. 651201 (Greiner − Longwood, FL)
- Clear flat bottom collection plates cat. 9017 (Corning-Costar − Acton, MA)
- Jouan CR312 centrifuge equipped with swinging bucket rotor with plate carriers
- MultiScreen vacuum manifold cat. MAVM0960R (Millipore Corp. − Billerica, MA)
- Vacuum/Pressure pump and tubing cat. XX5500000 and XX7100004 (Millipore Corp. − Billerica, MA)
- Mettler-Toledo balance
- Laboratory blender (Waring)

- SPECTRAmax® Plus UV/Vis microplate reader with software 4.1 (Molecular Devices − Sunnyvale, CA)

Protocol

1. Cell lysate preparation

Ten grams of chicken liver were blended with 120 mL of Lysis Buffer containing Protease Inhibitor Cocktail (1µl cocktail/ml of lysis buffer). The lysate was clarified by centrifugation at 7500 x g for 10 min, then aliquoted and stored at –20°C.

2. MultiScreen Ultracel-10 filter plate concentration procedure

Liver lysate was used undiluted or diluted 1:10 and 1:20 (with 50 mM Tris buffer pH 7.5). Three hundred microliters of diluted lysate or 200 µL of undiluted lysate were added to each well of the filter plate. The plates were either centrifuged at 2500 x g or filtered by vacuum at 18"Hg. The remaining concentrate volume was measured by weighing on the Toledo-Mettler balance.

3. Protein concentration

Protein concentration was determined by BCA Protein Assay (following the microwell plate protocol from Pierce) with a working range of 20 to 2000 µg/mL. Albumin standard was used as a standard. Absorbance was measured at 562 nm on the SpectraMax Plus plate reader.

Results

Protein concentration speed was compared using the MultiScreen Ultracel-10 filter plate in vacuum mode versus centrifugal mode. The initial protein concentration in the lysate was estimated to be 5.8 mg/mL. Figure 1 demonstrates retentate volume decrease over time and that 200 µL of the lysate can be reduced to 70 µL by centrifugation and to 115 µL by vacuum in 30 minutes.

In a second experiment, 300 µL of two different dilutions of the liver lysate (1:10 and 1:20) were concentrated in the MultiScreen Ultracel-10 filter plate by either centrifugation or vacuum. Figure 2 shows the retentate volumes at different time points. After 50 minutes of filtration 3X and 10X volume reduction was achieved by vacuum and centrifugation, respectively. Centrifugal ultrafiltration provided faster and more efficient volume reduction than vacuum filtration.

One of the most important factors in parallel protein concentration is reproducibility throughout the plate. Figure 3 shows reproducible filtrate collection in eight rows of the MultiScreen Ultracel-10 filter plate with both centrifugal and vacuum filtration. Gradual increase of total protein concentration is shown in Figure 4. It is demonstrated that efficient 3-6X concentration of cell lysate proteins can be achieved in the MultiScreen Ultracel-10 plate in both centrifugal and vacuum mode. While more consistent concentration by vacuum filtration was observed (see Figure 4 for standard deviation), centrifugal filtration achieves higher protein concentration in a shorter time.

Figure 1: Comparison of undiluted liver lysate volume reduction in MultiScreen Ultracel-10 filter plate using vacuum or centrifugal filtration.

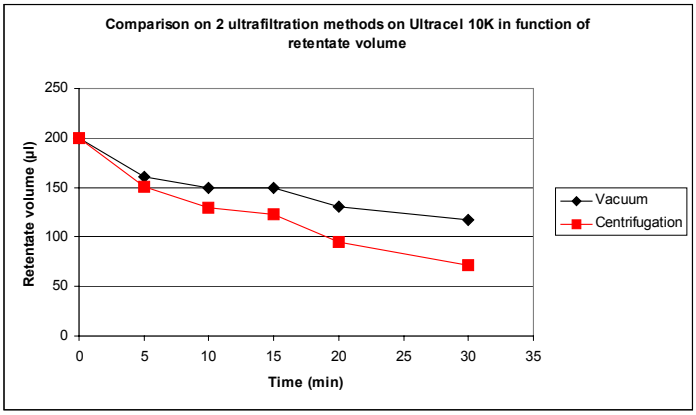


Figure 2: Liver lysate volume reduction during ultrafiltration in MultiScreen Ultracel-10 filter plate. Comparison of two filtration methods at two different lysate dilutions.

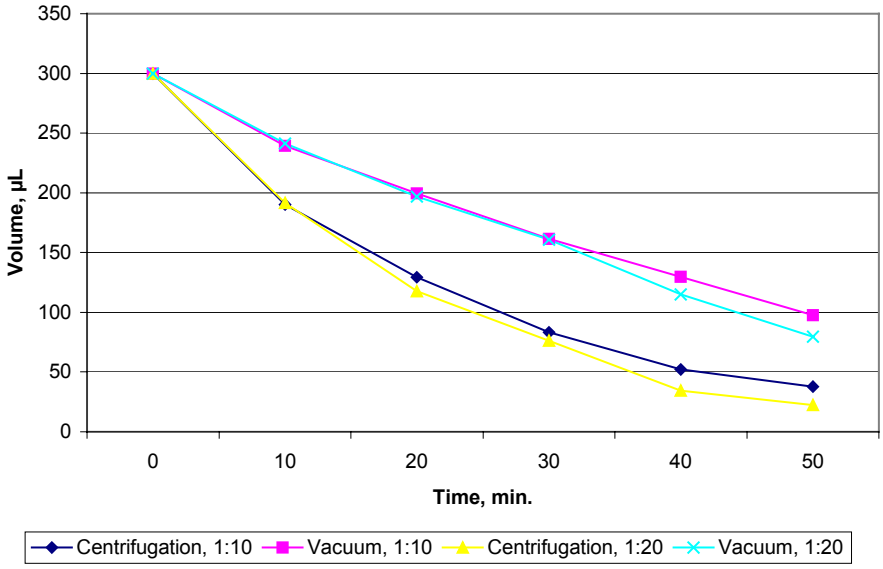


Figure 3: Well-to-well reproducibility of filtrate volume after 45 min filtration in MultiScreen Ultracel-10 filter plate using centrifugation or vacuum. Each bar represents an individual wells.

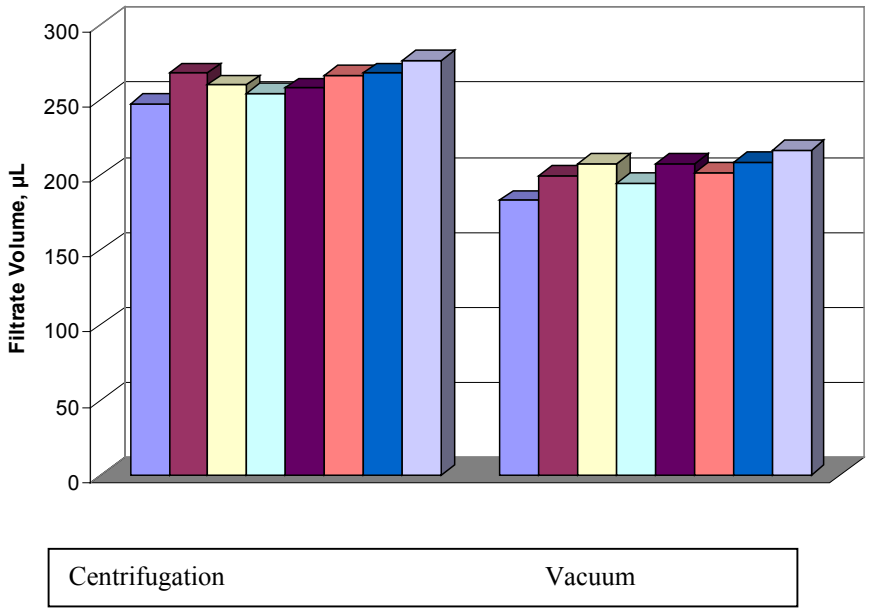
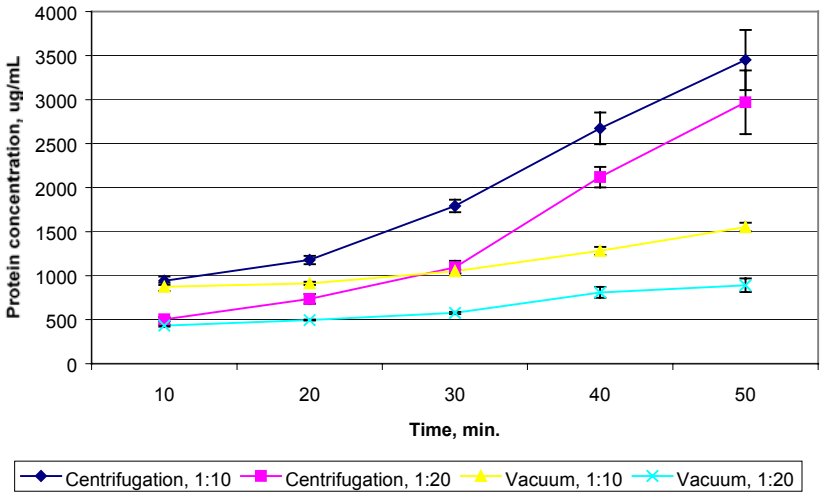


Figure 4. Protein concentration in the retentate as a function of time and cell lysate dilution. Each point represents an average of eight wells.



Part 2. Concentration of individual proteins within whole cell lysate

Objective:

Whole tissue lysates contain a complex mixture of proteins, peptides, nucleic acids and smaller molecules. Proteins characteristics including molecular weight, pI, hydrophobicity can vary as well. In the process of ultrafiltration, it is important that the relative ratio of proteins stays the same. This requires an equivalent parallel increase of the concentration of all the components of the protein mixture. In this study, the behavior of individual proteins during liver lysate concentration in MultiScreen Ultracel-10 filter plate was examined.

Method:

Reagents

- The same as in Part 1
- Liver lysate samples as prepared in Part 1 using the Multiscreen Ultracel-10 filter plate
- NuPage® NOVEX Bis-Tris 4-12% 1mm thick, 15-well SDS gels cat. NP0323 (Invitrogen − Carlsbad, CA)
- NuPage Sample Reducing agent (10X) cat. NP009 (Invitrogen − Carlsbad, CA)
- NuPage SDS Sample Buffer (4X) NP007 cat. (Invitrogen − Carlsbad, CA)
- SimplyBlue™ SafeStain Coomassie® G-250 stain cat. LC6060 (Invitrogen − Carlsbad, CA)
- Montage® In-Gel Digest$_{ZP}$ kit cat. LSKGDZP96 (Millipore − Billerica, MA)
- α-Cyano-4-hydroxycinnamic acid cat. 70990(Sigma − St. Louis, MO)
- HPLC grade Acetonitrile cat. A998SK-4 (Fisher Scientific − St. Louis, MO)

Equipment and materials

- The same as in Part 1
- HP ScanJet 4c/T scanner (Hewlett-Packard − Palo Alto, CA)
- Bio-Rad Molecular Analyst® scanner with Quantity One® software 4.2.3 (Bio-Rad Laboratories − Hercules, CA)
- Adobe® Photoshop® 5 software (Adobe Systems Inc. − San Jose, CA)

- Bruker Autoflex mass spectrometer (Bruker Daltonics − Billerica, MA)
- Xcell *SureLock*™ Mini-cell vertical electrophoresis system cat. EI0001 (Invitrogen − Carlsbad, CA)
- Mascot® search engine, http://www.matrixscience.com (Matrix Science Ltd. − London, UK)

Protocol

1. Separation of liver lysate proteins by SDS PAGE.
All liver lysate samples concentrated at different time points during vacuum or centrifugation (Part I) were prepared and separated in 4-12% gradient NuPage® NOVEX Bis-Tris gel, under reducing conditions according to the manufacturer's recommendations (Invitrogen). Gels were stained with Coomassie SimplyBlue and scanned in HP ScanJet 4c/T.
For densitometry analysis, gels were also scanned on BioRad Molecular Analyst and total volume per band was calculated using BioRad Quantity One 4.2.3 software.

2. Protein identification.
Protein bands were excised from Coomassie blue stained SDS PAGE gel and in-gel digested with trypsin according to Millipore protocol for the Montage In-Gel Digest$_{ZP}$ kit. Peptides were eluted from the ZipPlate® C18 plugs (included in the Montage kit) by centrifugation and spotted onto a Bruker MALDI target. Mass spectra were acquired in the reflector mode. All searches were done using Mascot search engine with mass accuracy of 100 ppm.

Results

Figure 5 follows the changes in protein concentration in the liver lysate over time during ultrafiltration in MultiScreen Ultracel-10 filter plate. The SDS-PAGE gel clearly demonstrates parallel increase of total protein concentration within 45 minutes of centrifugation. To confirm total protein concentration data obtained in Part 1, densitometry analysis was performed on the gel. Total optical density of each lane and the density of ten individual bands were analyzed. The proteins in those bands were identified by in-gel digest. The results of the densitometry study are shown in Figures 6 and 7. It is clear that all proteins analyzed behaved similarly in the process of ultrafiltration and that a parallel concentration of every protein in the samples was achieved. No proteins were lost to the membrane or into the filtrate.

Figure 5. SDS-PAGE gel of liver lysates concentrated with the MultiScreen Ultracel-10 filter plate at different time points. Five µL samples from each time point were loaded on the gel. Proteins identified by in-gel digestion are shown on the right.

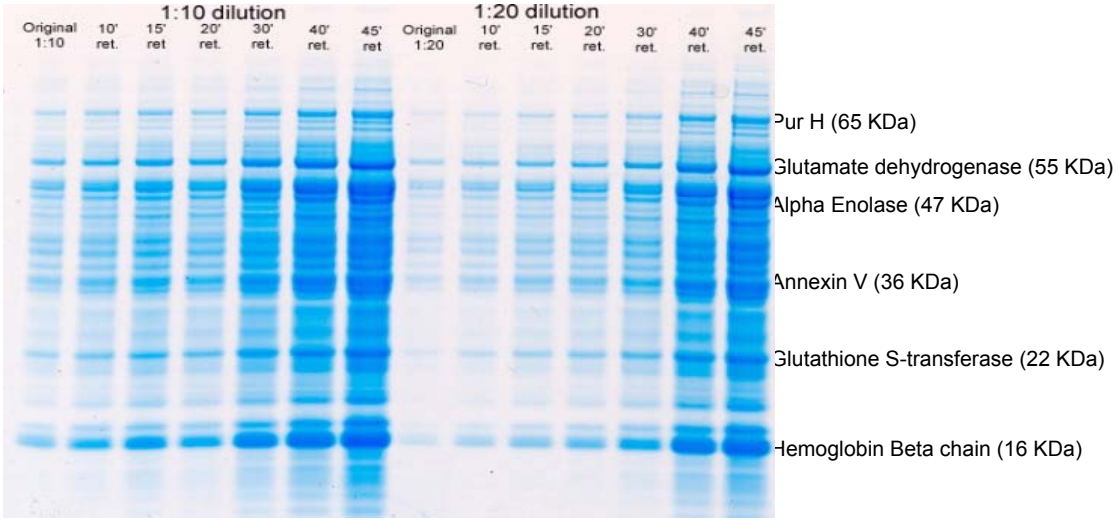


Figure 6: Densitometry analysis of the gel in Figure 5 by lane.

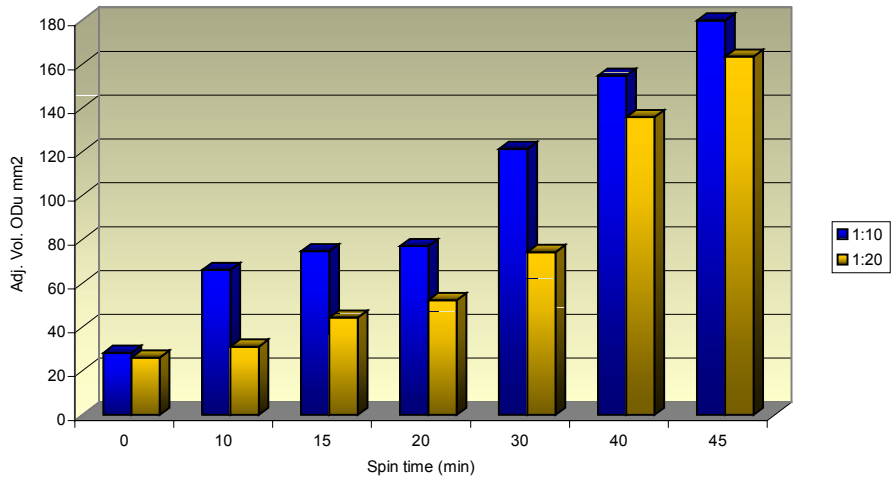
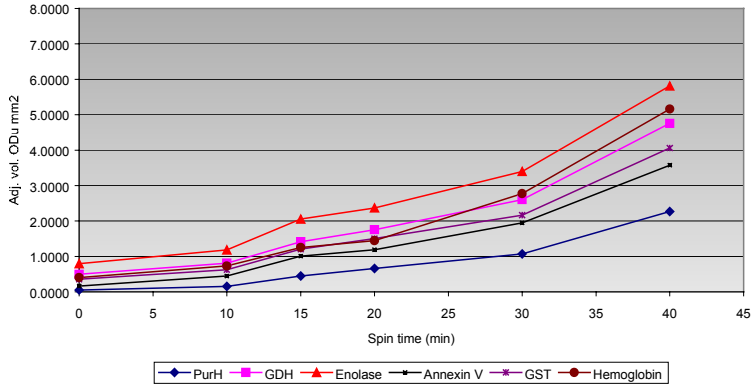


Figure 7: Densitometry analysis of the gel in Figure 5 (1:20 dilution) for six individual proteins



Related Protocol Note and Data Sheet

PC1025EN00	Protein Retention, Recovery, Volume Recovery, and Guidelines for Concentration and Desalting
PF2050EN00	MultiScreen Filter Plate with Ultracel-10 Membrane Data Sheet

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