

OVERVIEW

Polyacrylamide gel electrophoresis (PAGE) is an ideal technique for the separation of intact proteins. The Gelfree™ 8100 Fractionation System applies the principles of PAGE in a format that provides fast, preparative separations of protein containing samples for applications in proteomics. The system features include:

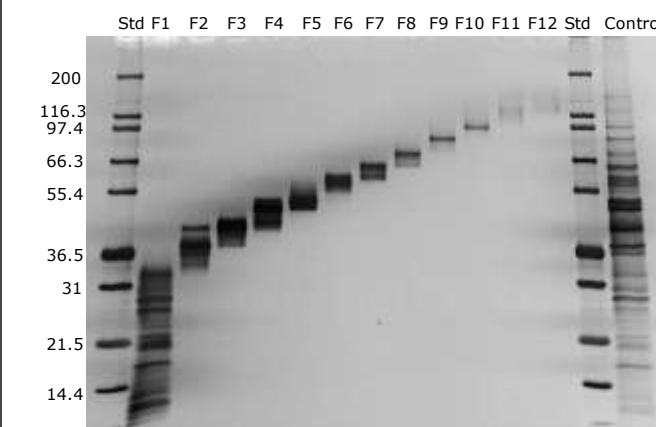
- Bench top instrument and single-use, eight channel cartridges
- Uses SDS-PAGE to solubilize and partition complex protein mixtures on the basis of molecular weight
- Fractions are recovered in liquid phase (rather than in gel)
- High load capacity (>500 µg per channel)
- Prepares 8 samples in 90 minutes
- Compatible with depletion, IEF, protein digestion, LC-MS, and western blot

This approach is versatile, equipping users with the capability to tune the separation and isolate specific molecular weight fractions. The system provides programmable, reproducible methods making it possible to repeat experiments and maintain quality data. The high loading capacity and high recovery of the system yield increased proteome coverage and enhanced resolution.

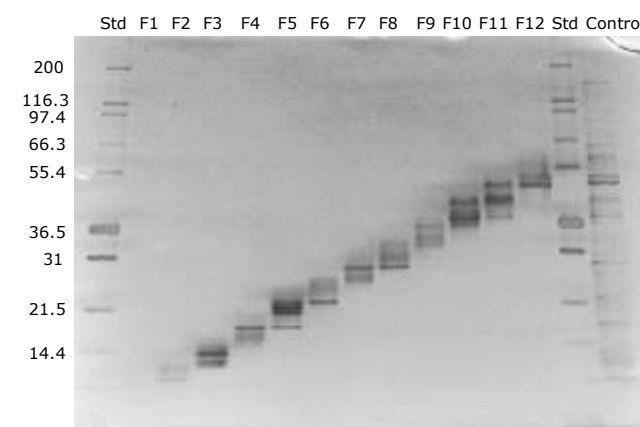
INTRODUCTION

The performance of polyacrylamide gel electrophoresis (PAGE) for the separation of intact proteins is unrivaled. The advantages include versatility, flexibility, and scalability. Literally dozens of commercial devices are sold that utilize this valuable tool for the separation and preparation of complex samples. Independent comparative studies have shown that PAGE fractionation yields the highest proteome coverage and reproducibility of any technique prior to LC-MS/MS. Recently, the technique of Gel-Eluted Liquid Fraction Entrapment Electrophoresis (GELFREE) was introduced. This application of SDS-PAGE combines this proven technology with a novel format and chemistry, permitting the rapid fractionation of proteins in a complex sample according to their molecular weight (Anal Chem, 80, 1568-1573). This study characterizes the performance of the technique according to four criteria: mass range, resolution, reproducibility, and recovery.

SYSTEM PERFORMANCE FOR PROTEIN FRACTIONATION



a) 8% Cartridge, 3.5-150 kDa



b) 12% Cartridge, 3.5-50 kDa

Figure 4: Fractionation of complex samples using the Gelfree 8100 System. Samples containing 500 µg of *S. Cerevisiae* cell lysate were fractionated using two different cartridges.

- 8% cartridges are used for proteins having a molecular weight between 3.5 and 150 kDa and provide optimal resolution between 30 and 150 kDa.
- 12% cartridges provide optimal resolution for protein molecular weights between 3.5 and 50 kDa.

An aliquot of each Gelfree fraction corresponding to 6% of the total collected volume (150 µL) was loaded into each lane of a 1D gel. Electrophoresis was performed and the results visualized by silver staining.

The availability of different cartridge types provides fractionation that is tailored to specific applications.

GELFREE 8100 FRACTIONATION SYSTEM



Figure 1. The Gelfree 8100 instrument (left) is an eight channel electrophoretic controller that supplies voltage independently to each of the eight channels in the pre-cast cartridge (right). A touch screen display located on the front of the instrument allows the user to program the voltage to apply to each channel, as well as the fraction collection intervals. To use the system, the user programs the sequence (or chooses from a pre-programmed sequence) for each of the eight electrophoretic channels and starts the experiment. The device automatically pauses the experiment whenever the time interval has expired, allowing the user to extract the molecular weight fraction of interest. Measurement information for the eight channels is displayed to the user in tabular and graphical format during the course of an experiment.

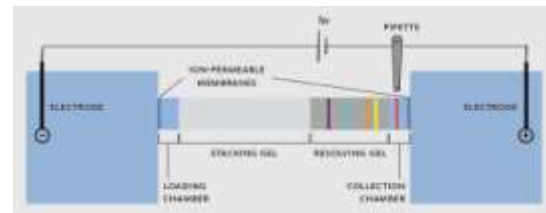


Figure 2: Schematic of the Gelfree device. The technology uses SDS-PAGE using specialty pre-cast composite gels to separate analytes based on molecular weight. As molecular weight fractions elute from the end of the gel, they are entrapped in a 150 µL liquid layer defined by the end of the gel and a molecular weight cut-off membrane.

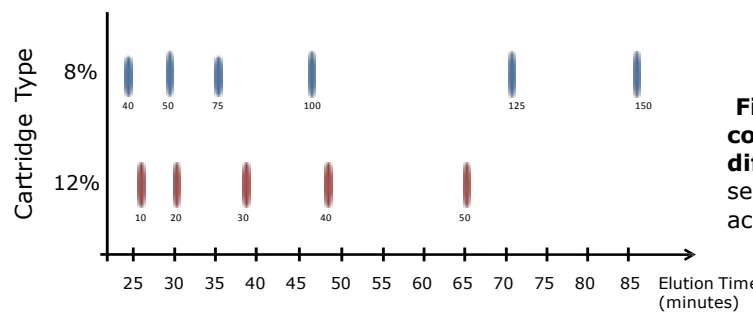


Figure 3: Different cartridge compositions are optimized for different mass ranges. Cartridges are selected to provide optimum resolution across the range of interest.

PERFORMANCE EVALUATION

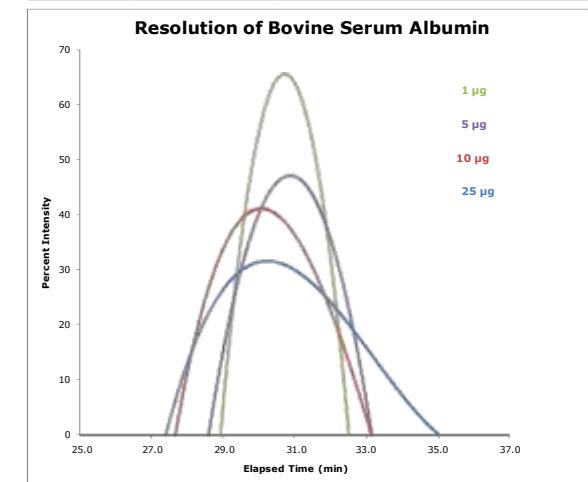


Figure 5: Resolution of separation as a function of protein load. Bovine serum albumin peak width as a function of protein load. Peak width varies between 2 and 7 minutes, FWHM. Width increases at higher loading. Fractions were collected at 90 second intervals to define the elution window.

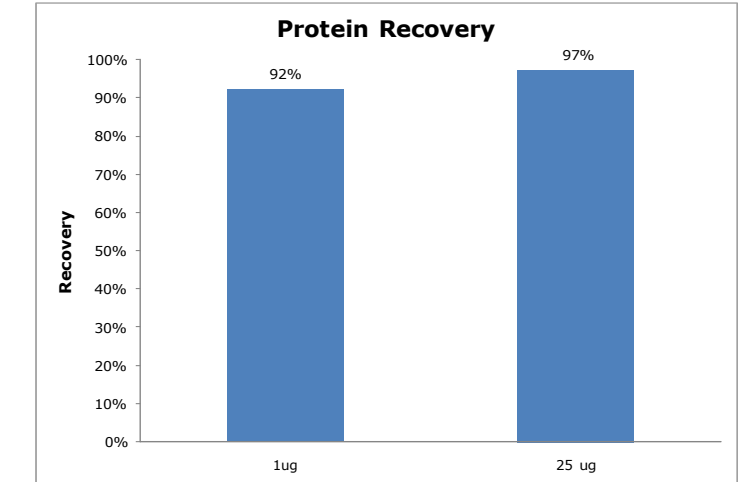
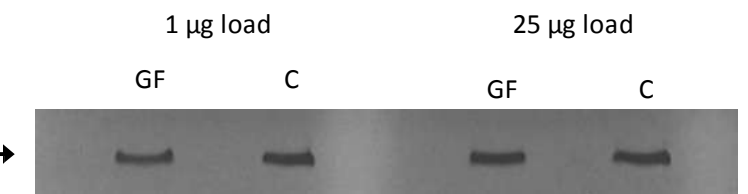


Figure 6: Optimization of recovery. Simple adjustment of the collection times allows for a protein to be collected in a single fraction at near quantitative recovery. Using the elution time data in Figure 5, collection times were optimized for maximum recovery. Gel image shows the Gelfree fraction (GF) containing albumin, 1 µg and 25 µg, compared to control.

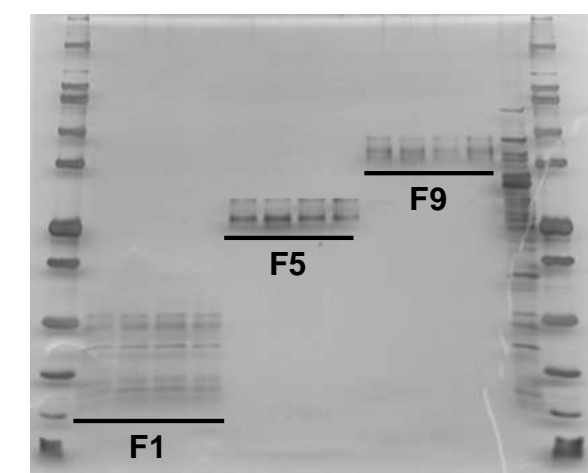


Figure 7: Gelfree 8100 provides reproducible fractionation. Brain extract was loaded onto the Gelfree 8100 at 200 µg/channel. Fractionation was performed according to the standard protocol. Three fractions from four adjacent channels are compared for reproducibility.

SUMMARY

Gelfree 8100 Fractionation System provides:

- Broad mass range analyte fractionation
- Separation based on molecular weight
- High loading capacity (>500µg per channel)
- High reproducibility (RSD <5%)
- Non-biased sampling of protein classes (hydrophobic, acidic, basic, LMW, etc.)
- Fractions recovered in liquid, rather than in gel
- Analytes recovered intact
- High analyte recoveries and in-solution digestion protocols increase MS data quality

Applications include:

- Discovery Proteomics
 - Top-down: fractionation
 - Bottom-up: fractionation, distinguish isoforms
 - Protein interaction analysis: complex purification
- Targeted Proteomics
 - Protein/peptide quantitation: fractionation, IP clean-up
 - Biotherapeutic characterization