

## TECHNICAL NOTE

### Cartridge Selection for the Gelfree® 8100 Fractionation System

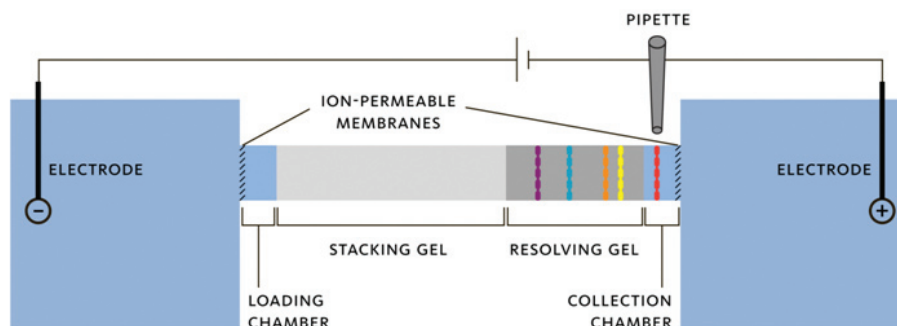
#### INTRODUCTION

The Gelfree 8100 Fractionation System is a gel electrophoresis-based protein fractionation tool that separates a complex protein sample into fractions according to molecular weight. Uniquely, the Gelfree 8100 System uses a polyacrylamide tube gel to perform the separation but delivers the eluted protein fraction in liquid phase, allowing for optimum recovery of the sample. Coupled with high loading capacity of the system, these advantages make the Gelfree 8100 the premier tool for protein fractionation in preparation for mass spectrometry analysis.

The type of gel chosen for the separation will affect the character and quality of fractionation. To provide versatility necessary to address multiple sample types and mass ranges, the Gelfree 8100 Fractionation System can be used with any one of four types of cartridge. These different cartridge types range in polyacrylamide percentage from 5-12%. This technical note reviews the performance of each of the cartridge types available and provides the information necessary to select the optimum cartridge type for your application.

#### PRINCIPLES OF GELFREE SYSTEM OPERATION

Figure 1 shows a schematic representation of a single channel of the eight-channel Gelfree cartridge. A tube gel is housed between two chambers having limited volume and bound by ion-permeable, molecular weight cut-off membranes. The sample is placed into the loading chamber and voltage is applied across the device. Sample proteins are separated according to their electrophoretic mobilities. As the proteins reach the end of the gel, they are eluted into the collection chamber where they can be collected using a pipette. The elution times and the resolution are affected primarily by the polyacrylamide medium through which they are separated. The percentage of the polyacrylamide can be selected to focus the separation on the range of protein molecular weights of most interest to the user.



**Figure 1: Schematic of the Gelfree 8100 Fractionation System.** The technology uses SDS-PAGE to separate analytes based on molecular weight. As molecular weight fractions elute from the end of the gel, they are retained in a liquid layer defined by the end of the gel and a molecular weight cut-off membrane.

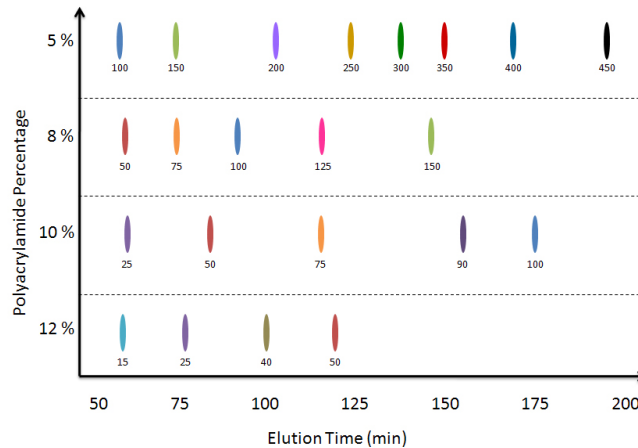
### UNDERSTANDING THE OPTIONS

Gelfree cartridges are available in four types: 5%, 8%, 10%, and 12% polyacrylamide. The schematic in Figure 2 compares the resolving properties of each cartridge type. All cartridges are constructed with membranes having a molecular weight cut-off of 3.5 kDa; therefore, even small proteins will be collected in each of the cartridge types. The primary differences among the cartridges is the molecular weight range across which the resolution is optimized and the maximum protein molecular weight that can be eluted from the gel.

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**Figure 2: Different cartridge gel compositions are optimized for different mass ranges.**

Cartridge is selected by the user to provide optimum resolution across the range of interest. Proteins eluting prior to approximately 50 minutes are not resolved and will be found in the first collected fraction. The labels under each band indicate the protein molecular weight in kDa.



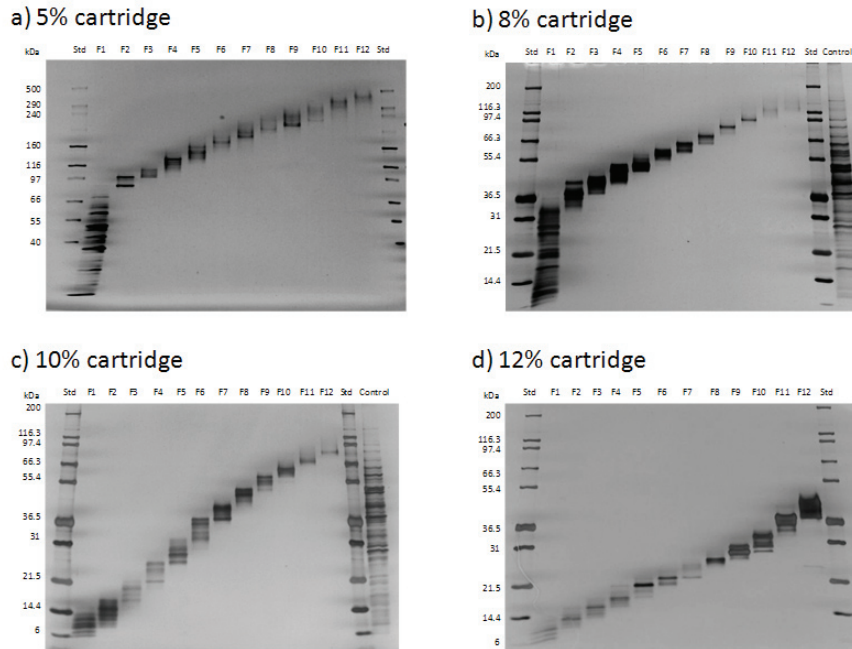
### MATCHING CARTRIDGE SPECIFICATIONS TO YOUR REQUIREMENTS

Table 1 provides guidelines for the selection of cartridges for protein fractionation using the Gelfree System. Proper cartridge selection is based on a number of factors. The primary consideration for cartridge selection is the molecular weight of the proteins of interest. For best results, the molecular weight range of interest should fall within the optimum molecular weight range for the cartridge selected. If this guideline is not adhered to, your proteins of interest may not be separated adequately, or they may not elute from the gel within the normal operation time of the cartridge chosen. If the sample molecular weight profile of the sample is not known, visualization using a 1D gel can guide the cartridge selection process. For general fractionation of whole lysates, it is recommended that the protein molecular weight distribution from the 1D gel analysis be matched as closely as possible to the optimum molecular weight range of the Gelfree cartridge. In some cases, multiple cartridge types may need to be employed to comprehensively fractionate the sample. To demonstrate the differences in separation provided by the different cartridges, Figure 3 presents the fractionation of a single yeast sample using each cartridge type.

**Table 1: Gelfree 8100 Cartridge Specifications**

% Polyacrylamide	MW Range	Optimum MW Range	Average Fraction Width
5%	3.5 - 500 kDa	75 - 500 kDa	40 kDa
8%	3.5 - 150 kDa	35 - 150 kDa	10 kDa
10%	3.5 - 100 kDa	15 - 100 kDa	7 kDa
12%	3.5 - 60 kDa	10 - 50 kDa	4 kDa

**Figure 3: Fractionation of yeast, *S. cerevisiae*, demonstrated using the four different Gelfree 8100 cartridges.** A 0.5 mg aliquot of yeast was separated into 12 different fractions using each of the four cartridge types. The resulting fractions were analyzed using tris-glycine 1D gels and visualized with silver staining.



### CONCLUSIONS

This technical note describes the strategy for selection among the four different types of cartridges for use with the Gelfree 8100 Fractionation System. The available cartridges provide a range of separation capabilities for proteins between 10 and 500 kDa. The selection of the cartridge is one of the most important steps in properly planning and executing the fractionation of a complex protein sample. The information provided here is intended to be used to aid in the selection process and provide the ideal cartridge for your application without extensive method development.

### ORDERING INFORMATION

To order the Gelfree 8100 Fractionation System or Gelfree 8100 Cartridge Kits, visit [www.proteindiscovery.com](http://www.proteindiscovery.com) to request a quote, or contact Protein Discovery, Inc. by phone, fax, or e-mail.

(T) 865.521.7400 / (TF) 866.670.9038 / (F) 865.521.3548  
[sales@proteindiscovery.com](mailto:sales@proteindiscovery.com)

DESCRIPTION	PART NUMBER
Gelfree 8100 Fractionation System	42001
Gelfree 8100 12% Cartridge Kit	42102
Gelfree 8100 10% Cartridge Kit	42105
Gelfree 8100 8% Cartridge Kit	42103
Gelfree 8100 5% Cartridge Kit	42104