expedeon
protein discovery & analysis

GELFREE® 8100
FRACTIONATION SYSTEM

TECHNICAL NOTE

Optimizing Molecular Weight Fractionation using the Gelfree® 8100 Fractionation System

INTRODUCTION

The Gelfree 8100 Fractionation System operates using the principles and methods of gel electrophoresis to carry out molecular weight-based separation and fractionation of complex samples. Just like conventional SDS PAGE, some common biological reagents, as well as other factors, can adversely affect performance of the separation, and lead to a variety of unwanted outcomes. This technical note describes some common problems encountered using the Gelfree 8100 system and offers suggestions to optimize the fractionation of real biological samples.

HIGH SALT

One of the primary contaminants found in biological samples is a high concentration of salt. Salt can be introduced into the sample through various means. Depending upon the manner in which a cell or tissue lysate is made and the ratio of tissue-to-lysis buffer used, the total amount of salt can vary significantly. The physiological levels of salt alone can be high enough to prevent proper operation of the Gelfree 8100 in certain samples. Additionally, frequently used cell lysis buffers are supplemented with salt to physiological levels. In conventional 1D gel electrophoresis, these samples can often be analyzed without consideration to the amount of salt present due to the fact that only a few microliters of sample are used. In a preparative experiment, such as the case with Gelfree 8100, the total volume of sample can be as much as 50-100 times that of an analytical gel.

The most common problem observed when samples have too much salt is change in elution time. When a high amount of salt is present, the conductance of the sample will be high, thereby reducing the field strength across the sample. The sample will be slower to load and elute from the gel than normal. In some cases, the sample will still stack properly and will separate through the gel. Collection of the fractions will lead to the separation of the proteins; however, adjustments to the fraction collection times will have to be made in order to avoid taking fractions before elution of the proteins has begun. Likewise, these samples often need to be run for an extended total time to reach the desired high molecular range. In severely overloaded samples, the sample may not load properly and the salt can prevent proper stacking of the sample, severely degrading the resolution of the separation.

GELFREE 8100 FRACTIONATION SYTEM

Sample desalting

When developing a protocol for sample desalting, an effective tool is a conductivity meter. To ensure the proper performance of Gelfree, the sample conductance after the addition of Gelfree 8100 Sample Buffer should be less than 5 mS cm^{-1}. For commonly used buffers such as Tris, concentrations less than 50 mM are acceptable depending on the other constituents of the buffer. For optimum reproducibility, predictable elution of proteins according to molecular

weight, and to ensure optimum resolution, removing the excess salt from the sample is critically important. There are a variety of methods useful for desalting protein samples. These include gel filtration columns, dialysis, and solid-phase extraction methods. The selection of method is dependent on the amount of sample to be processed. For most applications, desalting using a gel filtration spin column provides a convenient and effective solution to the problem. These desalting columns are sold in a variety of formats by numerous vendors. The type and number of buffers and salts that can contribute to sample conductance are too numerous to list on an individual basis.

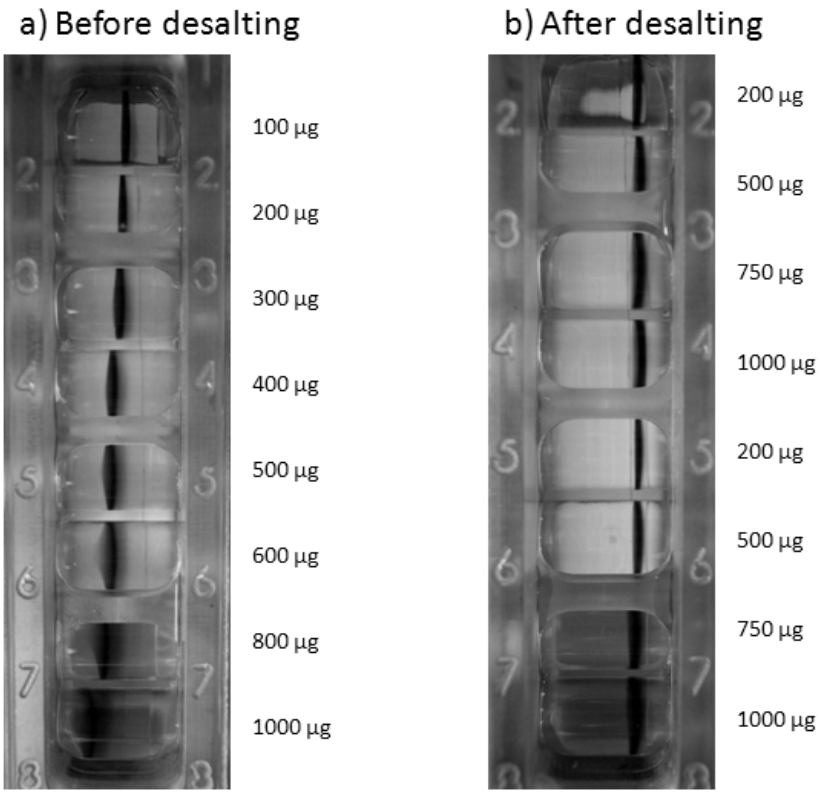

Figure 1: The effect of salt on the stacking and migration of proteins in Gelfree 8100. (a) Complex cell lysates prepared without desalting exhibit significant slowing of sample migration when more sample is loaded. Additionally, the presence of the salt creates distortion of the band, limiting resolution. (b) After desalting, the protein bands migrate uniformly with respect to time, regardless of the total sample loading. Furthermore, the elution times are normal.

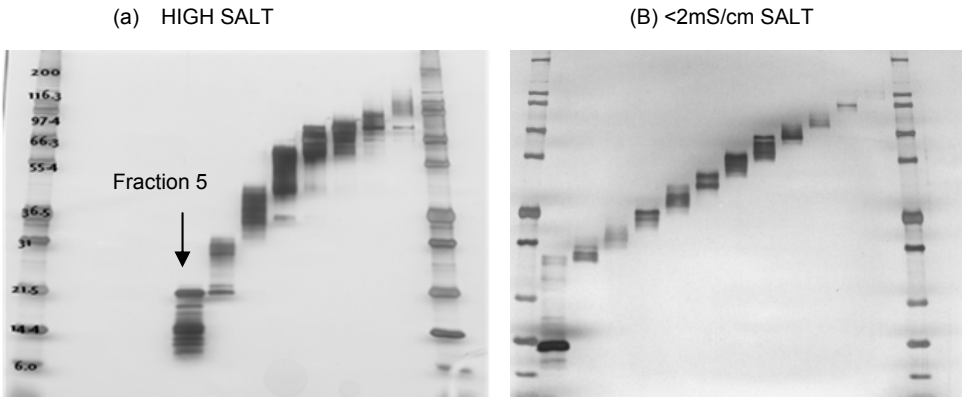

Figure 2: High salt results in later than expected elution of proteins. The example on the left (a) shows a protein extract prepared in phosphate buffered saline. The levels of salt found in PBS resulted in later than expected elution of the protein. As a result, the first four fractions collected contained no protein. The results from the 1D gel on the right (b) shows a typical result from protein extract run with the recommended amount of salt, <2mS/cm.

SAMPLE VISCOSITY

As with the presence of salt, the viscosity of the sample can have serious consequences on the separation. There are a number of naturally occurring biomolecules that can act to increase the sample viscosity. For example, the presence of high concentrations of polysaccharides, or the presence of nucleic acids in the lysed sample can result in a highly viscous sample. Examples of viscous reagents commonly mixed with biological samples include high concentrations of glycerol in the lysis buffer or sucrose from fractions taken from density centrifugation.

The equation for the electrophoretic mobility (u) of a protein under the influence of an electric field is

$$u = \frac{|z|e}{6\pi\eta r}$$

where z is the charge of the protein, η is the viscosity of the medium, and r is the radius of the molecule. This inverse proportionality to sample viscosity means that as the viscosity increases, sample migration rate decreases.

Symptoms of high viscosity

Often, the first indication of this problem will be that the sample is difficult to pipette due to its viscous nature. If this situation occurs, it is best to address the problem prior to fractionating the sample on the Gelfree 8100 system. In the case where the Gelfree separation were to proceed, one would observe significant delay in loading of the sample on the tube gel, loss of stacking efficiency of the sample, and loss of resolution. Depending on the viscosity, the effect can be quite severe. Figure 3 demonstrates the separation of a bacterial sample containing a polysaccharide contaminant that increases sample viscosity. In this example, proper selection of the method of sample preparation was necessary for proper separation of the sample.

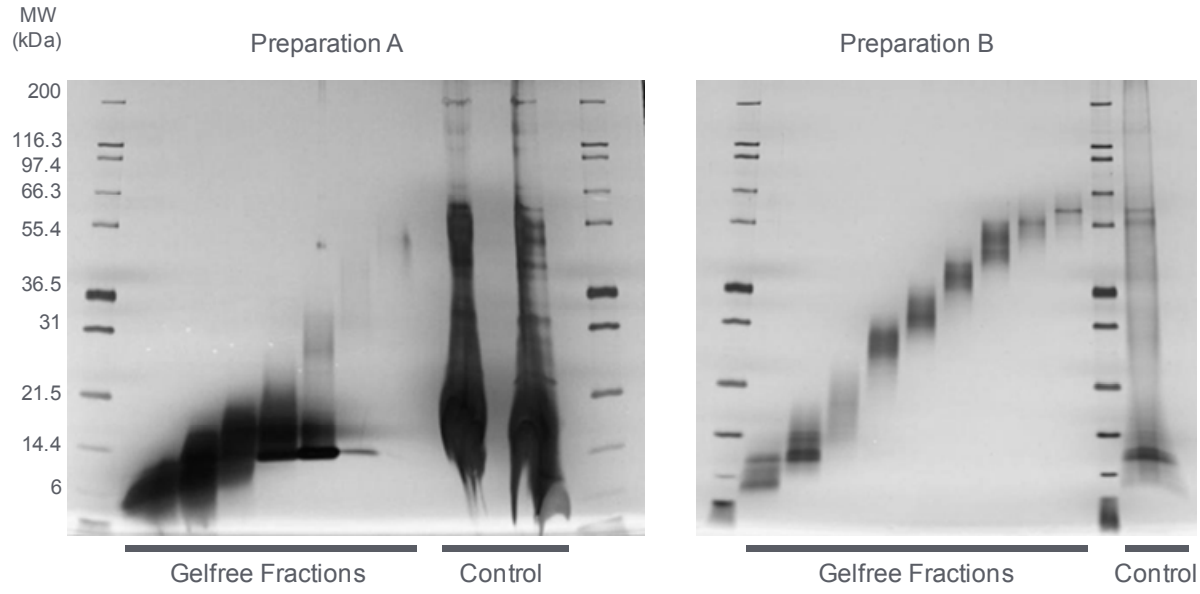

Figure 3: Sample viscosity negatively affects the protein separation using Gelfree 8100. This example shows a protein extract from identical bacterial samples prepared using two different lysis procedures. The sample contains an extracellular polysaccharide that is highly viscous. Preparation A originated from a bacterial lysis followed by protein precipitation. The carbohydrate contaminant is enriched with the protein, resulting in poor separation. Preparation B consisted of a wash of the cells to remove the polysaccharide contamination, followed by lysis of the cell. Note: Sample collection times are not identical for each preparation.

Reducing viscosity

Procedures for reducing high sample viscosity vary depending on the contaminating species. For low molecular weight contaminants such as sucrose and glycerol, often a simple dialysis or wash of the sample through an appropriate molecular weight cut-off membrane will adequately remove the contaminating species. If, however, the contaminating species is high molecular weight, a more situation specific strategy may be necessary. For samples contaminated with nucleic acids, the use of DNAse and RNAse are recommended.

EXCESS DETERGENTS AND CHAOTROPES

The molecular-weight based separation in the Gelfree 8100 system depends on the interaction between the detergent, SDS, and protein to carry out the separation. Ironically, an excess of detergent or the presence of species that alter the way in which SDS interacts with the protein can affect performance. One must carefully select the lysis buffer to minimize these problems. Figure 4 shows an example of a yeast cell lysate prepared in 8% SDS. The result is that the sample fractionation is poor with a high likelihood that a single protein elutes across multiple fractions.

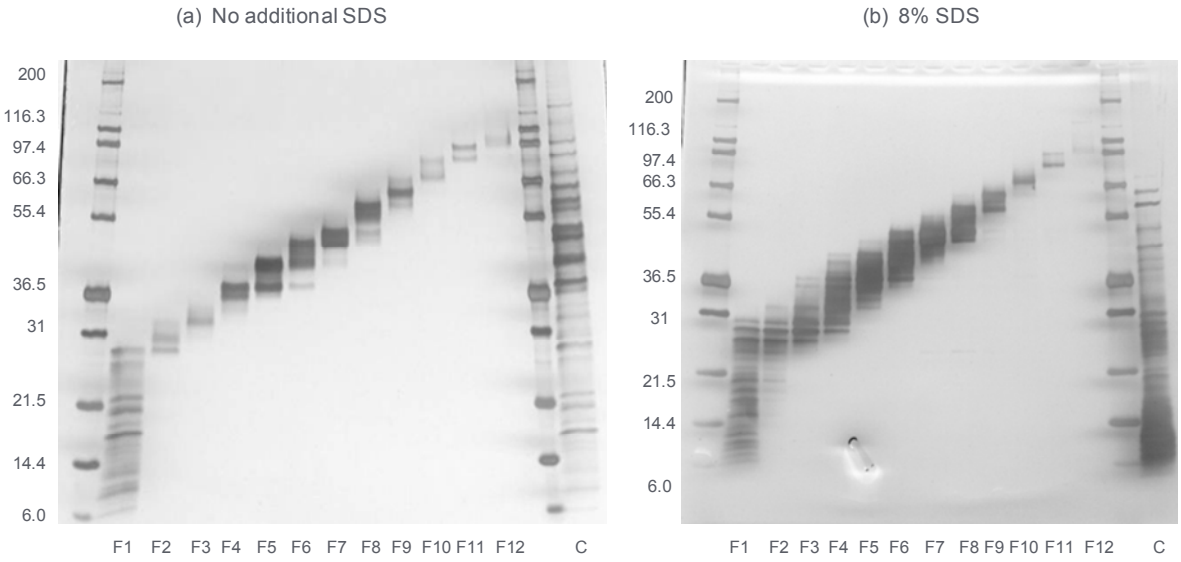

Figure 4: Excess detergent affects Gelfree 8100 performance. This example shows a protein extract from *S. Cerevisiae* prepared using (a) YPX lysis buffer and (b) a lysis buffer containing 8% SDS. The impact on the separation is two-fold. The excess conductance introduced by the charged SDS resulted in elution of the proteins later than expected. In this example, the fraction collection times were adjusted from the normal recommended times. The second effect is band broadening and loss of resolution.

Ideally, one would eliminate detergent and chaotropes from the sample prior to loading the sample on the Gelfree 8100 system; however realizing that these reagents are necessary in some cases, one must minimize their presence as much as possible. Avoid the use of ionic detergents other than SDS. When SDS must be used to solubilize the sample, limit the concentration to less than 2.5%. If other detergents must be used, select from among the many non-ionic detergents available for use. Urea is widely known to interfere with 1D gel electrophoresis and should be avoided if possible.

INSOLUBLE MATERIAL

Insoluble material in the sample can cause multiple problems in the gel. Often, the problem results in sample streaking. The insoluble material can block the gel pores, thus increasing temperature in the tube gel. As the separation continues, often some of the insoluble material can become soluble. The result is poor or no discernible resolution. Samples should always be clarified by centrifugation prior to loading in the Gelfree 8100.

SAMPLE pH

The sample pH has an important part in determining the charge on the protein in solution; therefore, significant deviations from the operating pH of 8.4 can cause the protein migration to be affected. For best results, the sample pH after mixing with Gelfree 8100 sample buffer should be within 1 pH unit of pH 8.4. Strongly buffered solutions or acidic protein precipitations such as TCA precipitation commonly result in protein solutions that may significantly differ from the recommended range. Deviations in resolution and elution time will result.

PROTEIN EXTRACTION

The UPX Universal Protein Extraction Kit (#44101) has been designed to maximize protein recovery during tissue and cell lysis and is fully compatible with the Gelfree 8100 fractionation. Similarly, the YPX Yeast Protein Extraction kit (#44102) is recommended for yeast protein extraction when preparing samples for use with the Gelfree 8100 Fractionation system.

The use of similar commercial products may yield higher conductivity extracts that will require a desalting step prior to loading in the Gelfree 8100 system.

PREPARING THE SAMPLES FOR MS ANALYSIS

Following fractionation using the Gelfree 8100 system, the samples may be prepared for MS analysis using one of two recommended methods. If intact proteins are to be analyzed, then the SDS may be removed from the fractions using Pierce Detergent Removal Spin Columns (Thermo, #87777). Alternatively, the detergent, salts, and other contaminating species may be removed from the samples followed by trypsin digestion using a FASP Protein Digestion Kit (Protein Discovery, #44250).

CONCLUSIONS

This technical note describes ways to optimize fractionation of a complex sample using the Gelfree 8100 Fractionation system by elucidating some of the most common interferences and suggesting alternate procedures.

For a complete list of troubleshooting scenarios, please refer to the Tips to Improve Resolution guide at www.ProteinDiscovery.com or contact Protein Discovery, Inc. Technical Support at 865.521.7400.

GELFREE 8100 CARTRIDGE KIT

ORDERING INFORMATION

To order the Gelfree 8100 Fractionation System or Gelfree 8100 Cartridge Kits, visit 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) 865.521.35348
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

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