GELFREE® 8100 Fractionation System

Frequently Asked Questions

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INDEX

FREQUENTLY ASKED QUESTIONS

Basics

Q. What does the GELFREE® 8100 Fractionation System do?

A. The GELFREE® 8100 System fractionates complex protein mixtures into liquid-phase molecular fractions. It's like being able to cut out any number of bands in a 1-D and extract them into solution, but without slab gels and cutting, and with much higher recoveries. And, proteins are recovered intact, so you can analyze post-translational modifications or tie the intact protein molecular weight to the identified peptide in bottom- up mass spectrometry experiments.

Q. Is protein recovery affected by pI or hydrophobicity?

A. No. Molecular weight fractionations using the GELFREE® 8100 System are unbiased by protein pI or hydrophobic character.

Q. What is the buffer composition?

A. GELFREE® cartridges use a specially designed HEPES-SDS buffer system.

Q. What is the pH of the running buffer?

A. The pH of the running buffer is 7.8.

Q. Do the sample and running buffers contain SDS?

A. Both the sample buffer and the running buffer contain SDS.

Q. What is the gel?

A. The GELFREE® 8100 System uses proprietary polymer gels that have been specially designed for maximum resolution, reproducibility, and robustness.

Q. How many times can you use a GELFREE® cartridge?

A. Each of the eight channels in a cartridge is an independent system which can be used once. If you have one or two samples for fractionation, you can process just those samples using the GELFREE® 8100 System without using up a whole cartridge. The unused channels will still be available for use at another time.

Features

Q. What is the throughput of the system?

A. A typical run time is two to three hours to fractionate up to eight samples simultaneously over the entire mass range of 3.5 kDa to 500 kDa.

Q. How long does it take to fractionate proteins?

A. If the only proteins of interest are of low molecular weight, a fractionation can be finished in just a few minutes. If multiple molecular weight fractions are being collected, or if high molecular weight proteins are targeted for fractionation, run times are longer.

Q. What is the mass range for protein fractionation?

A. The GELFREE® 8100 System enables liquid-phase protein fraction collection from 3.5 kDa to 500 kDa.

Q. How much protein is recovered?

A. Total recovery will depend on the protein of interest. For albumin (MW 66 kDa), recovery was determined to be >90 %.

Q. What is the reproducibility for the quantity of recovered proteins?

A. The GELFREE® 8100 System is highly reproducible. For example, the CV for the amount of albumin recovered through the GELFREE® system was determined to be 8.4% (n=8).

Q. What is the typical mass resolution?

A. The mass resolution is 5-10% of molecular weight of the fraction (%CV). At 50 kDa, the range is approximately 7-10 kDa. For optimum resolution within a particular range, select a cartridge for which the range of interest is at or near the center of the optimum range specified for the cartridge. In other words, select a cartridge where the range of interest is near fraction 6 when using the method outlined on the Quick Reference Card. For more information, please refer to our Technical Note on cartridge selection.

Q. What is the advantage of the GELFREE® technology over size exclusion chromatography?

A. The GELFREE® system provides a wider mass range (from 3.5 kDa to 500 kDa), better yield, and, in many instances, better resolution. With multiple-channel, parallel throughput, the GELFREE® system provides faster results and does not require an LC column, so you don't have to dedicate an LC system or switch it back and forth for different applications.

Sample Loading

Q. How do I prepare cells for protein fractionation on the GELFREE® System?

A. Ideally, use serum-free media for cell culture. This will help avoid contamination of the sample with albumin.

For protein extraction from cells, we recommend our UPX™ or YPX™ Universal Protein Extraction Kits (#44101, #44102). These products are compatible with the GELFREE® System and outperform other commercially available lysis buffers by a wide margin. For starting amounts, we do not recommend a specific number cells; rather, we have based our use instructions on a wet pellet mass. This measure is generally applicable to a variety of cell types. As you gain experience with the cell types you are fractionating, you can substitute another starting material measurement method if you desire.

We recommend also that you use a protease inhibitor mixture, such as our Proteoloc™ Protease Inhibitor Cocktail (#44201), to protect extracted proteins from degradation by endogenous proteases.

For quantification, we recommend our Proteoquant™ Proteome Quantification Assay Kit (#44110), which was specifically designed to be compatible with reducing agents and detergent used for efficient extraction and fractionation.

Q. Can I simply add Acetate Sample Buffer (5X) and reducing agent to my sample, or do I need to lyse my sample prior to fractionation on the GELFREE® System?

A. Better results are obtained if the samples are lysed and (if necessary) desalted.

For sample lysis, we recommend our UPX or YPX Universal Protein Extraction Kits (#44101, #44102). These kits are compatible with the GELFREE® System and deliver an abundance of unbiased protein for fractionation and analysis.

Q. What is an acceptable salt concentration for a sample prepared for fractionation on the GELFREE® System?

A. For fractionation to proceed similarly to those described on the Quick Reference Cards, sample conductivity should be < 2 mS/cm. For sodium chloride, this translates to a salt concentration of approximately 20 mM.

If your sample is already at a low conductivity, there is no benefit to desalting.

If the sample conductivity is > 2 mS/cm, desalting it will reduce conductivity to < 1 mS/cm and result in more consistent, higher quality separations. If you choose not to desalt a sample with a conductivity > 2 mS/cm, be aware that the sample elution time will be increased. Samples can be run in this manner, but one should not expect the fraction times to correspond with those on the Quick Reference Card.

Q. What is the loading volume?

A. The sample loading volume is 1 – 112 µL per channel. Total loading volume is 150 µL per channel. The difference between the sample loading volume and the total loading volume is the Sample Loading Buffer.

Q. What is a typical sample loading amount (µg) for one channel?

A. If a sample contains only a few protein components, the loading amount should be no less than 1 µg per channel and no more than 25 µg. For complex samples, typical loading amounts range up to 500 µg total protein per channel.

Q. What happens if you overload the sample loading chamber with too much protein?

A. The resolution of the separation will deteriorate. The maximum loading amount depends on the sample, but we generally suggest loading up to 500 µg of total protein per channel. To fractionate 2 mg of total protein, you'll get the best results by loading 500 µg of protein into each of four channels.

Q. Can sucrose or glycerol be present in a sample prepared for fractionation on the GELFREE® System?

A. If the concentration of sucrose or glycerol is sufficiently low, and the sample does not sink to the bottom of the sample loading chamber, then it is acceptable. At high concentrations, the solution can become viscous, causing the elutions times to be later than expected.

Q. Can urea be present in a sample prepared for fractionation on the GELFREE® System?

A. High concentrations of urea can prevent proper denaturation and association of the SDS with the protein in your sample. If possible, it is best to avoid the use of urea. Expedeon recommends using our UPX or YPX Universal Protein Extraction Kits (#44101, #44102) for best results.

Q. Can I use a sample buffer that is typically used for 1D SDS-PAGE?

A. The GELFREE® Sample Buffer provided with the cartridge kit has been specifically designed for GELFREE® fractionation. For best results, we only recommend use of this buffer.

Operation

Q. How do I run fewer than eight channels and preserve the remaining channels for later use?

A. Carefully peel off the plate sealer. For the channels to be used, follow the protocol outlined in the User Manual. For the remaining unused channels, the storage buffer should be left in place and those channels should not be energized during the run. In other words, de-select the appropriate channels on the Channels Page. At the conclusion of the experiment, empty the running buffer from the used channel(s) and replace the plate sealer. Store at room temperature.

Q. Can you apply different fractionation methods to each lane?

A. No. The GELFREE® System is designed to run one method at a time.

Q. If voltage is applied to the wrong lane, will that lane become unusable?

A. If the channel that was energized contained storage buffer, running it for less than one minute should be OK. However, if the reservoirs contained running buffer, the trailing ion will have entered the gel ahead of the sample, making the lane unusable. Similarly, storing running buffer in an unused lane makes the lane unusable, as the buffer diffuses quickly into the gel.

Q. What is the maximum recommended voltage?

A. The recommended range is 50 – 100V. Running at 85V for protein applications results in the highest level of reproducibility.

Q. Do I really need to wash the sample loading chambers at the first pause?

A. No. However, if high amounts of protein are present in your sample, it is possible that some protein may have adsorbed to the walls of the sample loading chamber. This will result in a small amount of streaking through the gel. Our recommendation is to perform a single, quick wash of the sample loading chamber(s) with 150 µL of running buffer after the first fraction. After the wash step, add 150 µL of running buffer to the sample loading chamber, and 2 mL of running buffer to the cathode reservoir before resuming the method.

Q. When filling the reservoirs or adding the samples, I sometimes notice that bubbles can become trapped in the wells. Is this a problem?

A. Bubbles can be a source of irreproducibility and can at times prevent the samples from running all together. Anything that obstructs, or partially obstructs, the channel will change the electric field and create differences in elution time. Expedeon suggests careful pipetting so as not to introduce bubbles. If bubbles have been introduced, they may be easily dislodged by pipetting solution up and down before the run begins.

Q. One of the wells turned red during the run. What is the problem?

A. There are multiple reasons why a well would shut off during the run. This is indicated by the well indicator on the touch screen turning red and the voltage set to 0 volts.

1) There is no current passing through the well. Check for bubbles.

2) Wells that deviate from the average current of all running wells significantly will be turned off. This can happen if all 8 channels are running, but only one contains sample. The well that has been loaded can fail because the sample causes the current/voltage profile to differ from the average current/voltage calculated for the other 7 wells. This situation can be avoided if the unused wells are turned off.

Fraction Recovery

Q. How many fractions can you collect per channel?

A. A single molecular weight fraction may be collected containing the proteins of interest, or the sample may be partitioned into more than two dozen fractions. However, the user should keep in mind that the upper mass limit of the gel may be approached by running the sample approximately sixty minutes longer than the recommended 12-fraction method from the Quick Reference Card.

Q. Your examples describe the collection 12 fractions. Can I change the method to collect fewer fractions?

A. The system is user-programmable such that you can take as few or as many fractions up to 29, over whatever size range you desire. Keep in mind that whatever your fractionation method, the reservoir buffer should be changed every 45 minutes of continuous operation.

Q. What is the volume of eluted fractions?

A. The volume of each eluted fraction is 150-200 µL.

Q. What is the minimum collection volume?

A. A volume of 150 µL is the minimum volume. If a lower volume is desired, use a vacuum centrifuge to concentrate the fraction after collection. This will also increase the concentration of HEPES, Tris and SDS.

Q. Do high molecular weight proteins elute in more dilute fractions?

A. The GELFREE® 8100 System is designed specifically to limit high molecular weight protein diffusion by confining the eluted protein in a fixed volume rather than a stream of flowing buffer. The system can efficiently fractionate proteins up to 500 kDa with minimal dilution. However, longer fraction intervals will produce slightly increased fraction volumes.

Q. What happens to proteins above the high MW specification of the cartridge?

A. They remain in the gel.

Q. What happens to proteins below 3.5 kDa?

A. They are not retained in the sample collection chamber and migrate through to the anode reservoir.

Q. What percentage of SDS is contained in the fractions?

A. After the first fraction, the SDS concentration should be approximately 0.1%. The first fraction may contain slightly more SDS (up to 0.2%) due to the additional SDS contribution from the sample buffer.

Q. How should fractionated samples be stored?

A. Fractions may be stored overnight at 4 °C. If you need to store your fractions for more than one day prior to analysis, keep them frozen at -80 °C until you are ready to use them.

Tips

Q. For proteins prepared from crude samples by TCA precipitation, is there any recommended protocol for sample dissolution and preparation prior to fractionation on the GELFREE® System?

A. If you are fractionating the entire pellet, you can simply add 30 µL of Acetate Sample Buffer, 8 µL 1M DTT reducing agent, and 112 µL water. If only a portion of the pellet will be used, dissolve it in 10 mM Tris acetate buffer at neutral pH. Then add 30 µL of Acetate Sample Buffer, 8 µL of 1M DTT reducing agent, your sample, and water to 150 µL. When analyzing a TCA precipitation, it is always advisable to check the pH of the reconstituted solution to ensure that it is not acidic (>7.0). This will prevent proper operation of the GELFREE®.

Q. After I complete a fractionation experiment with GELFREE®, what chemical species remain in the sample?

A. The fractions collected from GELFREE® contain SDS (0.2% or less), Tris and HEPES (50 mM or less), and EDTA (1mM or less).

Q. How can I get rid of the SDS?

A. Numerous high recovery methods exist to remove SDS. However, our FASP Protein Digestion Kit (#44250) was critically tested and found to provide the highest reproducibility and recovery of any commercially available method – that's why we developed the product. In addition to removing SDS, the FASP Protein Digestion Kit simultaneously digests the protein samples in a high yield format. However, if you need your fractionated protein intact, we recommend the spin columns from Thermo Pierce (#87777).

Q. How can I get rid of salt?

A. If your sample is extremely salty, desalting will improve the reproducibility of your separations. Expedeon recommends using Amintra™ Desalting Spin Columns (ADS0050 and ADS0100) to eliminate this issue.

Q. What is the protocol for visualizing fractions produced by the GELFREE® System on a 1D SDS-PAGE gel?

A. To produce the gel images such as those appearing on the GELFREE® Cartridge Quick Reference Cards, mix a 5 µL aliquot of the GELFREE® fraction you wish to analyze with 8 µL water, 5 µL LDS sample buffer (Expedeon, NXB31010), and 2 µL 1M DTT. Heat for 10 minutes at 50 °C (Caution: excessive heating will cause protein degradation and streaking on the 1D gel), then load 10 µL of the heated solution onto a 15-lane gel. For fractions produced using the GELFREE® 12% Cartridge Kit, use a 4-12% Teo-Tricine gel (Expedeon, NXG41212). For fractions produced using other GELFREE® Cartridge Kits, use a 4-20% Teo-Tricine gel (Expedeon, NXG42012). Run the gel at 120V for approximately 2 hours, and then visualize proteins using our InstantBlue™ Coomassie Stain or by silver staining.

Q. How should GELFREE® Cartridges be stored?

A. GELFREE® Cartridge Kits are packed at room temperature and are shipped in an insulated container to avoid exposure to extreme temperatures during transit. Store cartridges at room temperature. Do not refrigerate or freeze the cartridges.

Troubleshooting

Q. Why is one lane running more slowly than the others?

A. Occasionally a bubble may become trapped against the membrane separating the sample loading chamber from the gel. When you add running buffer to the reservoirs, do so slowly so as to minimize the chances of producing a bubble. Inspect the membranes in each active channel prior to your run. If any bubbles are evident, gently free them from the membrane with your pipettor.

Q. How can I reduce high-abundance protein carryover?

A. High-abundance proteins should not appear in multiple fractions. If you see such carryover, this effect can be minimized. Empty and wash the sample loading chambers twice with 150 µL running buffer at the first pause then add 2 mL of running buffer to the cathode reservoirs. This removes high-abundance proteins which may have associated with the walls of the sample loading chambers. If the problem persists, check to ensure that you are not exceeding the maximum loading capacity or that your sample does not contain highly abundant proteins.

Q. Can I run a sample in an expired cartridge?

A. Expedeon does not support the use, and makes no claims regarding the performance, of a cartridge that is beyond its stated expiration date.

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