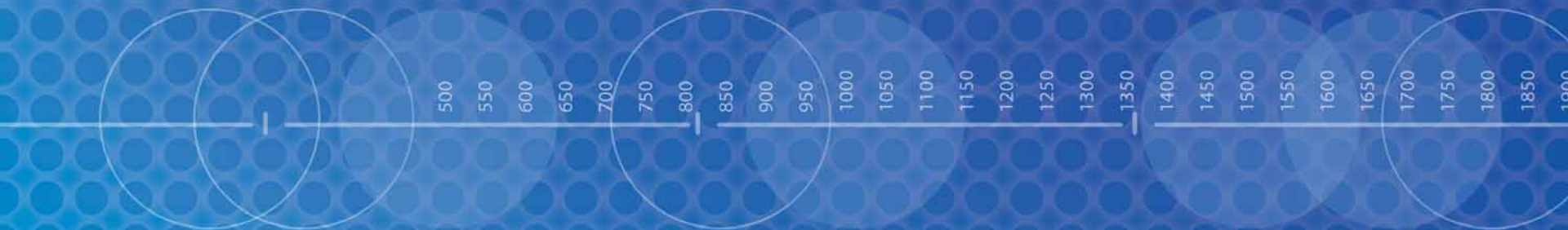


INNOVATIONS IN SAMPLE PREP



PRODUCT OVERVIEW

- GELFREE™ 8100 Fractionation System
 - Molecular weight-based fractionation with liquid phase recovery
 - Compatible with orthogonal fractionation methods such as IEF and reverse-phase HPLC for downstream analysis via MS or Western
- PPS Silent® Surfactant
 - Acid cleavable zwitterionic surfactant
 - Disrupts cell membranes, solubilizes hydrophobic proteins
 - No mass spec interference or greasy residue



GELFREE™ 8100 Fractionation System

- Molecular Weight Partitioning with Liquid Phase Recovery -

GELFREE™ 8100
FRACTIONATION SYSTEM



INTRODUCING
GELFREE™ 8100 FRACTIONATION SYSTEM



THE GELFREE TECHNOLOGY

- Gel-Eluted Liquid Fraction Entrapment Electrophoresis
- Uses SDS-PAGE to solubilize and partition complex protein mixtures on the basis of intact molecular weight
- Fractionation over the mass range 3.5kDa – 300kDa
- Fractions are recovered in the liquid phase with a pipette
- 8 samples in 90 minutes
- Compatible with depletion, IEF, protein digestion, LC-MS, and western blot

SYSTEM COMPONENTS



GELFREE 8100
FRACTIONATION STATION



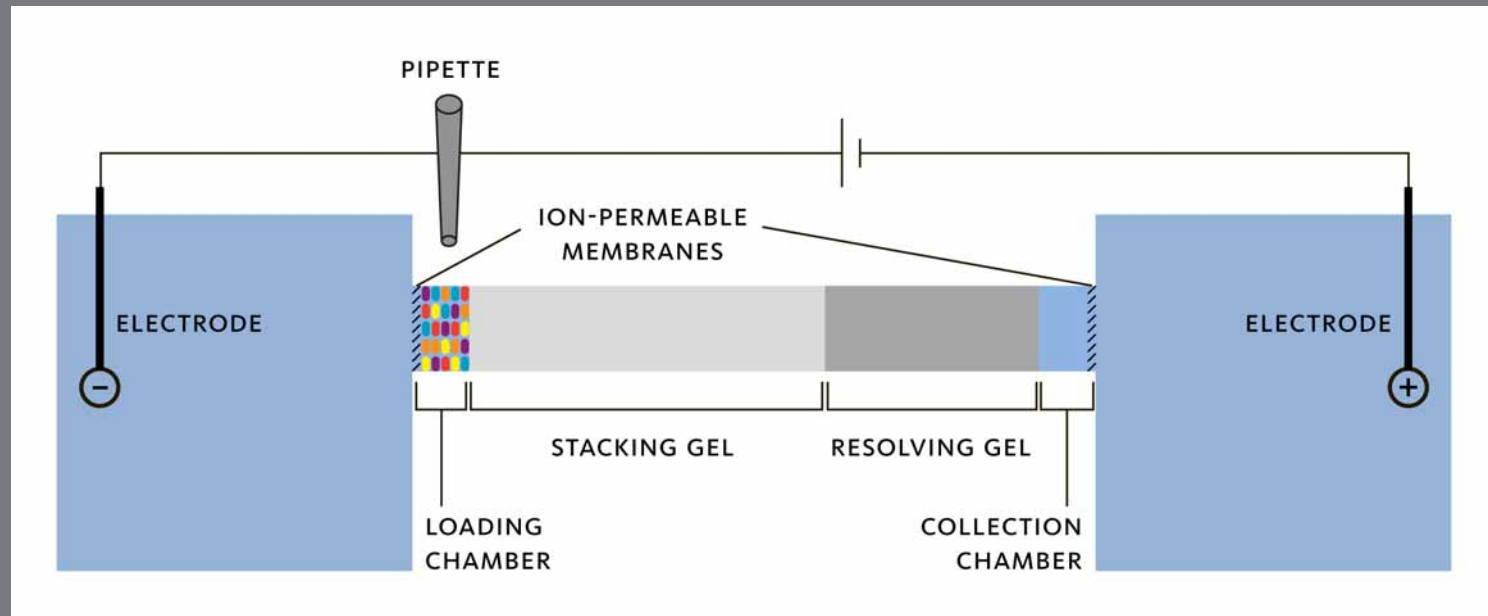
GELFREE 8100
CARTRIDGE

GELFREE 8100 CARTRIDGE KITS

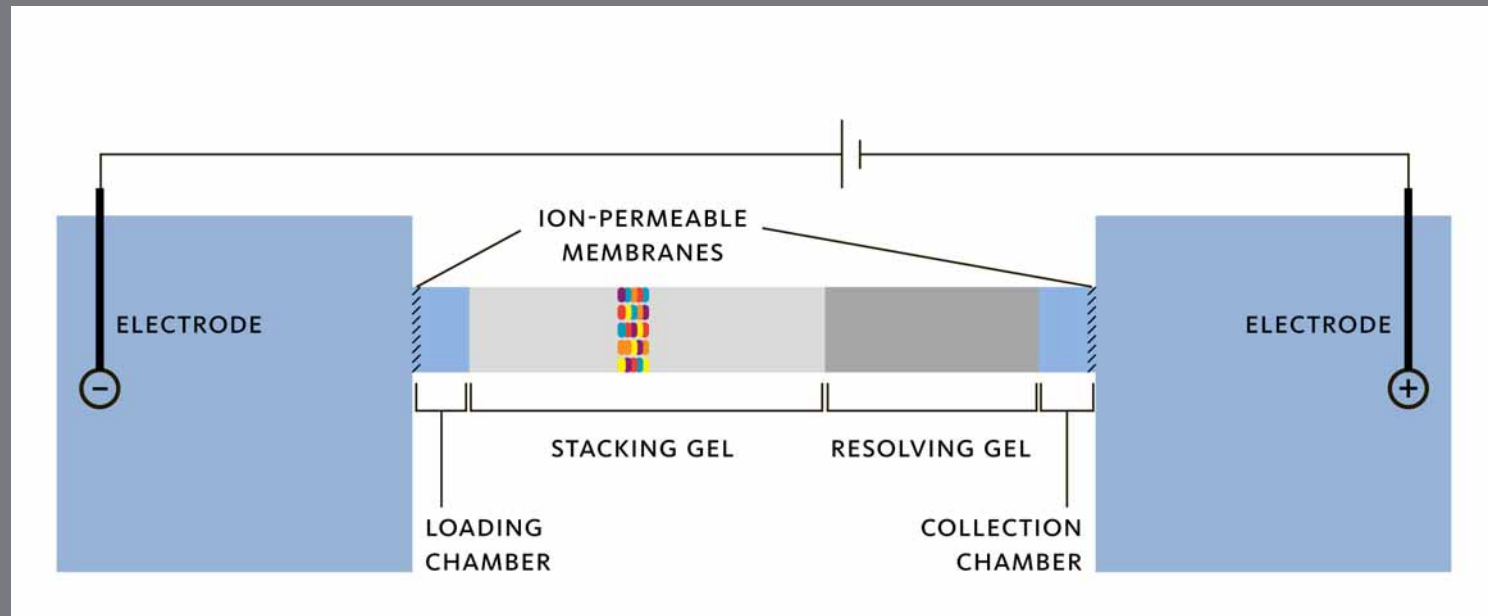
- *Low Mass* Cartridge Kit: 3.5 – 60 kDa
 - 12% Tris-Acetate Cartridge
 - Tris-Acetate Sample Buffer
 - MOPS Running Buffer
- *Mid Mass* Cartridge Kit: 30 – 150 kDa
 - 8% Tris-Acetate Cartridge
 - Tris-Acetate Sample Buffer
 - HEPES Running Buffer
- *High Mass* Cartridge: 60– 300 kDa
 - 5% Tris-Acetate Cartridge
 - Tris-Acetate Sample Buffer
 - Tricine Running Buffer



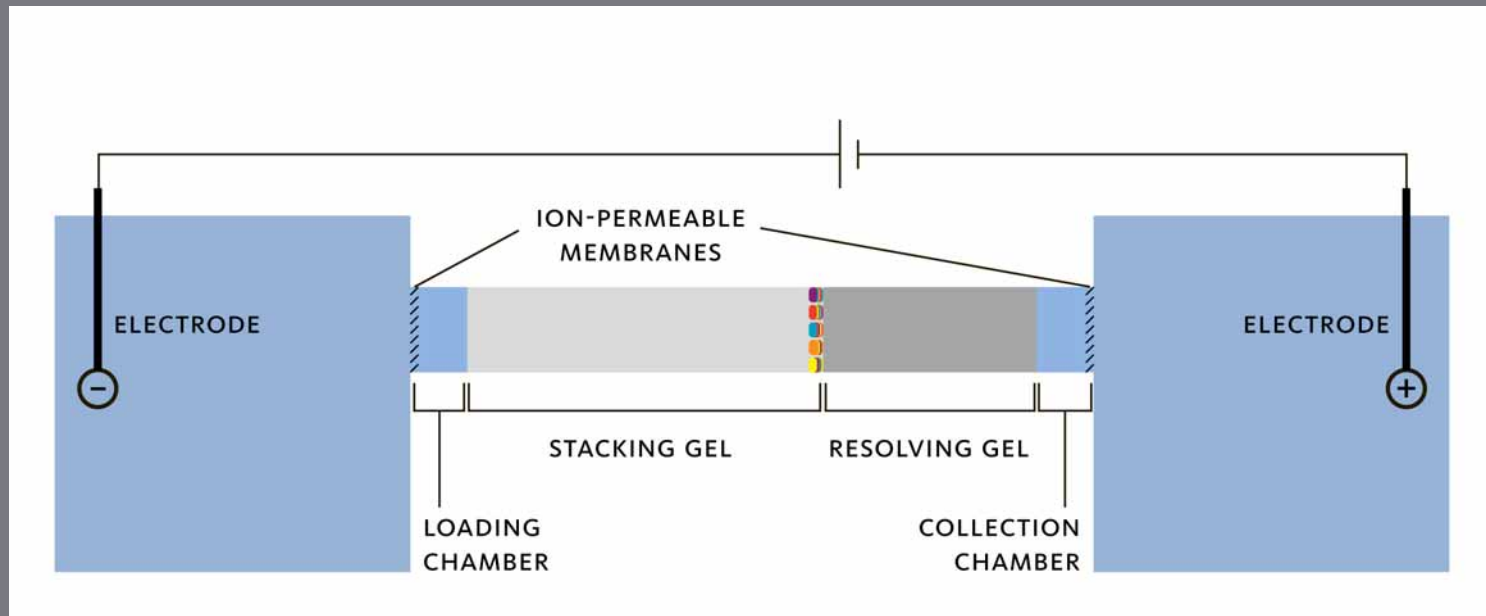
HOW IT WORKS



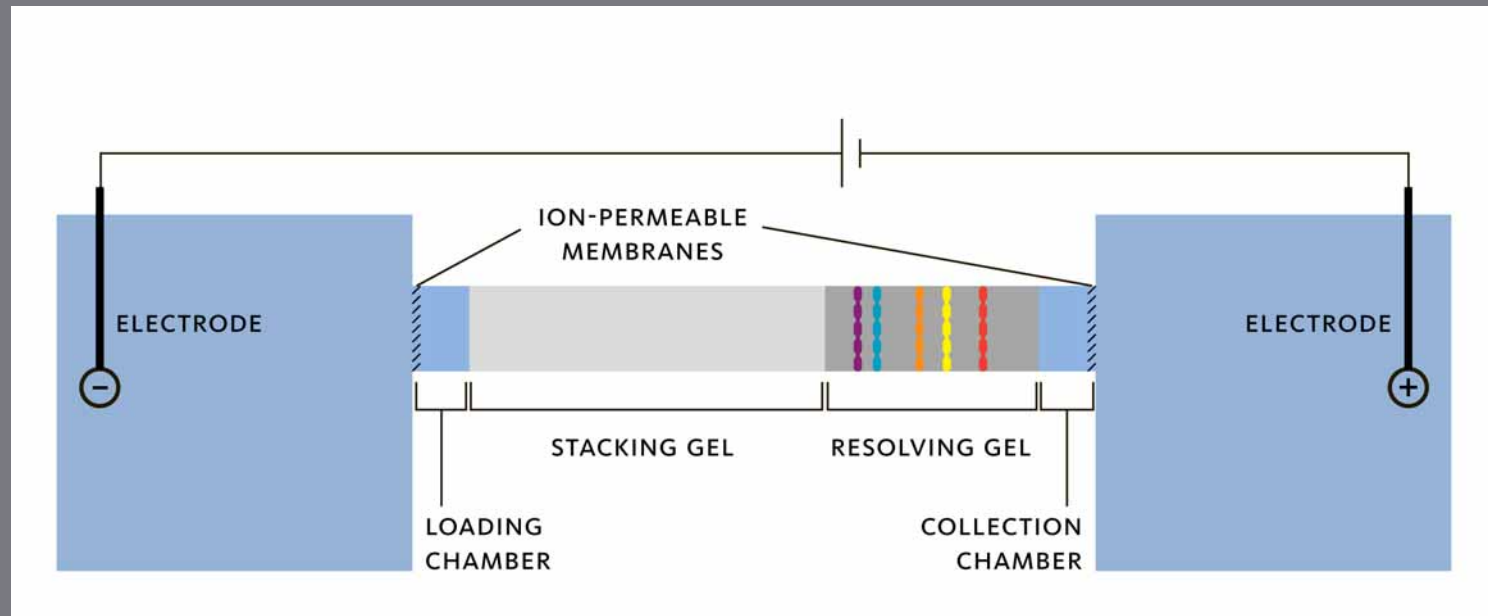
HOW IT WORKS



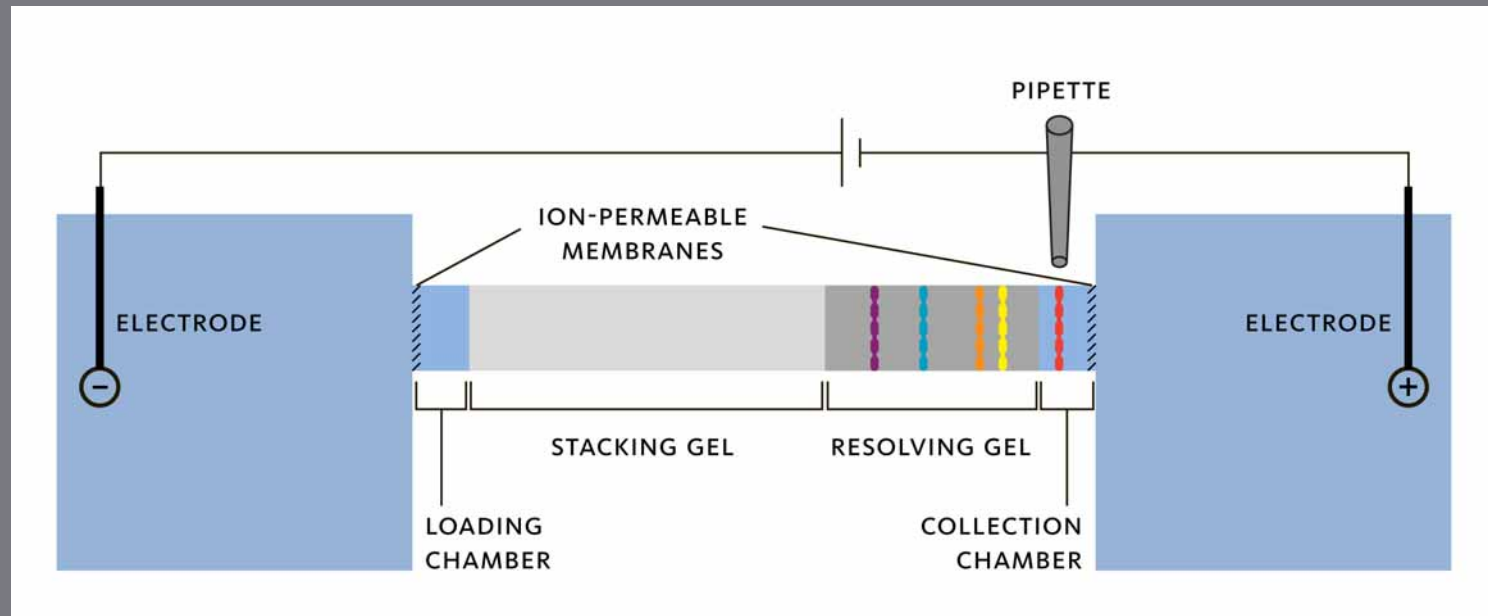
HOW IT WORKS



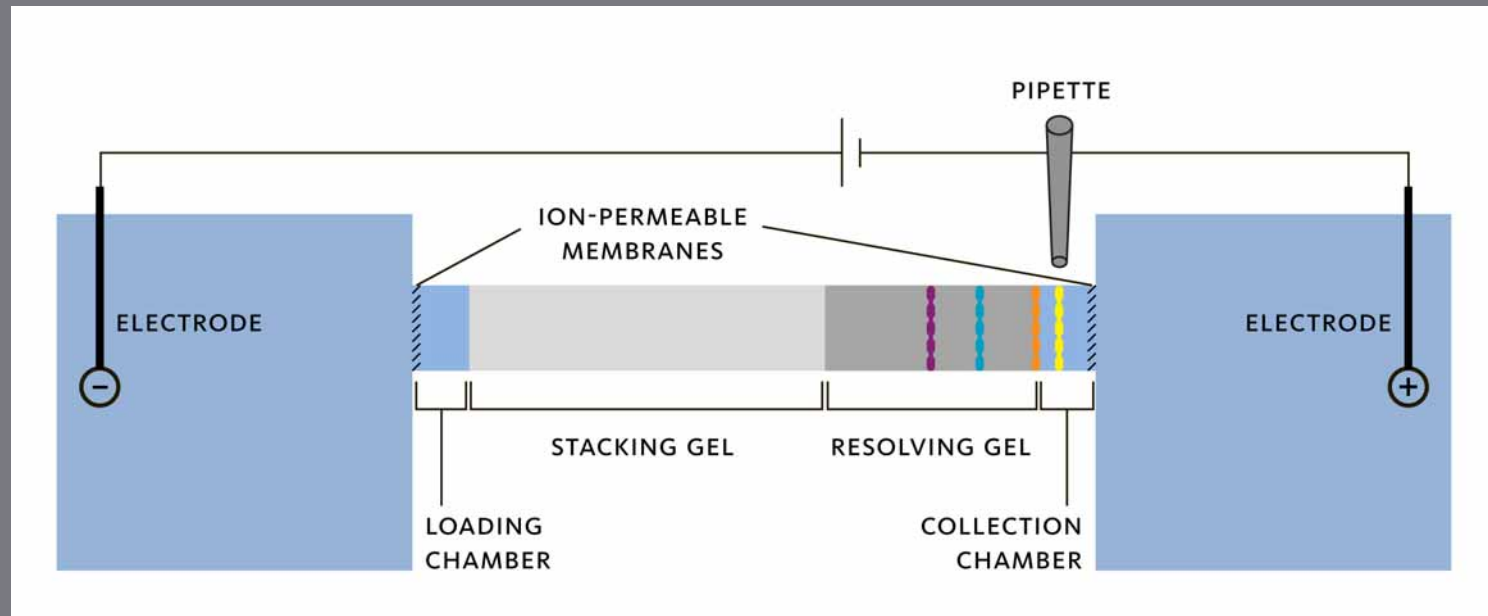
HOW IT WORKS



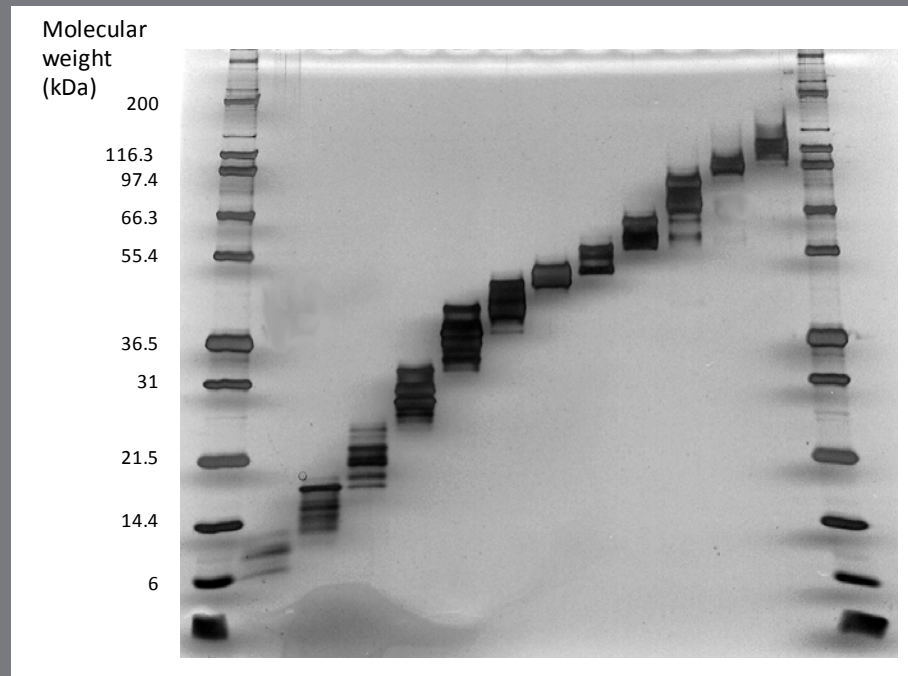
HOW IT WORKS



HOW IT WORKS

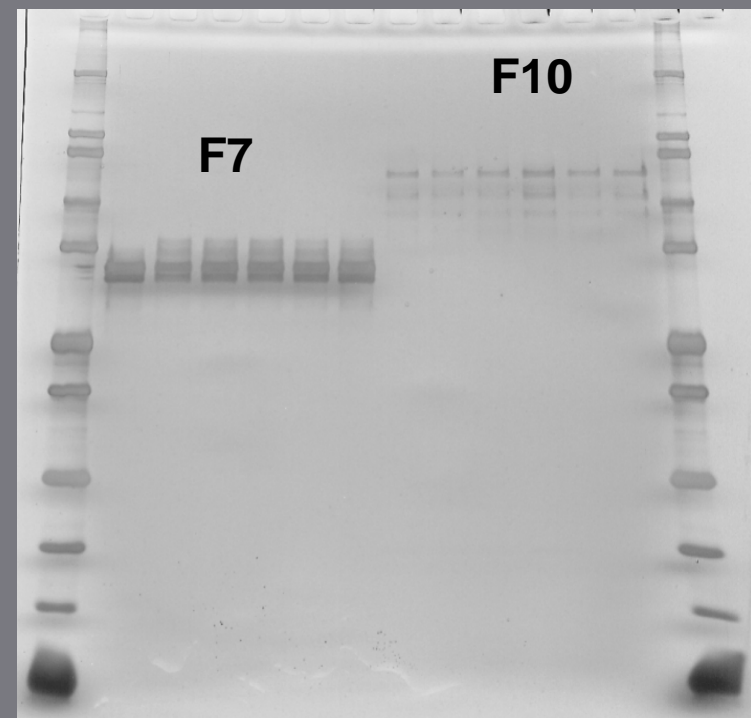
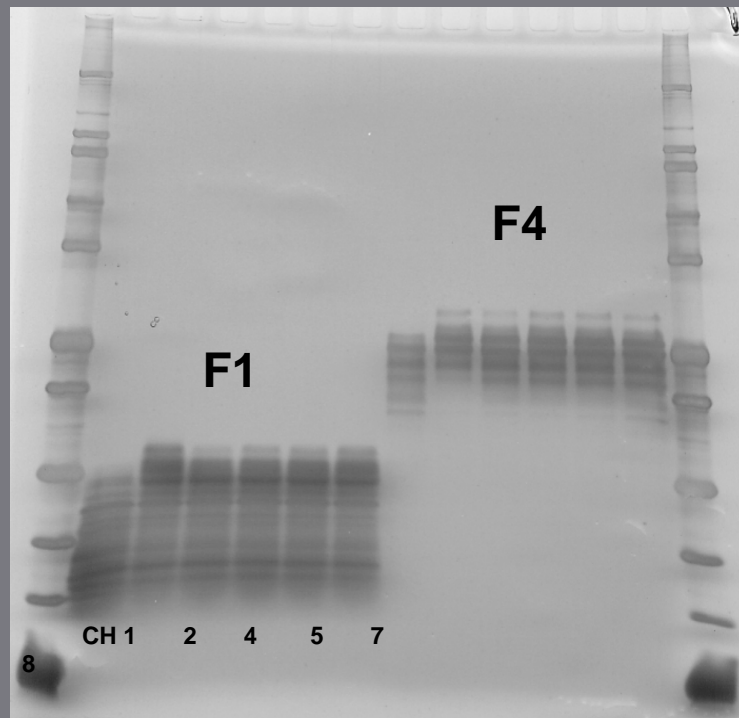


FRACTIONATED YEAST HOMOGENATE



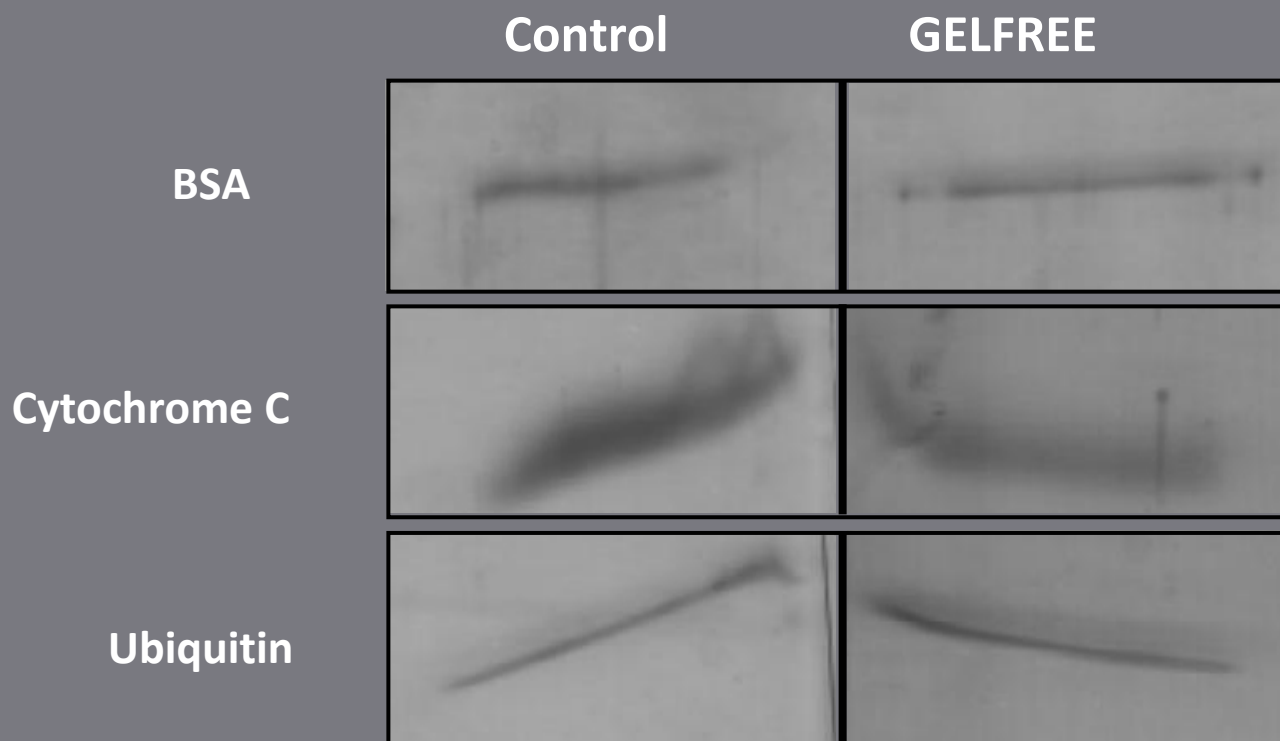
S. cerevisiae prepared using Gelfree 8100 Fractionation System. A 200 µg aliquot of yeast was fractionated into 12 fractions using the Gelfree 8100 system. 1D gel analysis with silver staining was used to visualize the results.

FRACTION REPRODUCIBILITY



S. cerevisiae extract is loaded onto the GF 8100 at 200 ug/channel. Fractionation is performed according to the standard protocol. Four fractions from 6 of the 8 channels are compared for reproducibility.

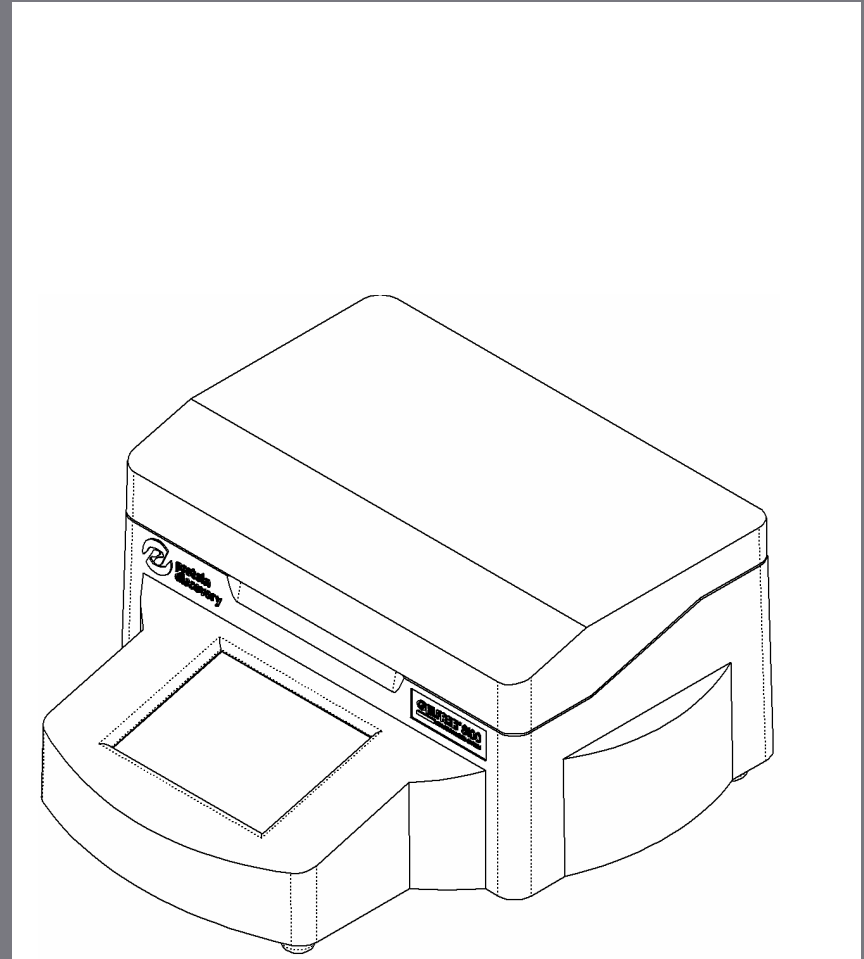
MINIMUM LOADING AMOUNT



40 ng shown above (500 ng into GELFREE)

RUNNING THE EXPERIMENT

- Load Cartridge
- Insert Cartridge
- Lower Electrodes
- Close Lid
- Program Method
- Run



PROGRAMMABLE FRACTIONATION

1	<input checked="" type="radio"/>	85 V	14.8 mA
2	<input type="radio"/>	85 V	14.6 mA
3	<input checked="" type="radio"/>	85 V	14.7 mA
4	<input checked="" type="radio"/>	85 V	14.8 mA
5	<input type="radio"/>	85 V	14.9 mA
6	<input checked="" type="radio"/>	85 V	14.8 mA
7	<input checked="" type="radio"/>	85 V	14.9 mA
8	<input checked="" type="radio"/>	85 V	14.8 mA

Time Elapsed

00:00:40

Selected Method: 1 Step: 1 of 12 (85 V, 1 min) Lid

Method 2

Step	Voltage	Time (Min:Sec)
1	Step 1	
2		
3	Voltage (V)	85
4	Time (min)	1
5		
6		
7	<input type="button" value="Prev"/>	<input type="button" value="Next"/>
8		
9		
10	<input type="button" value="OK"/>	<input type="button" value="Cancel"/>

ADVANTAGES

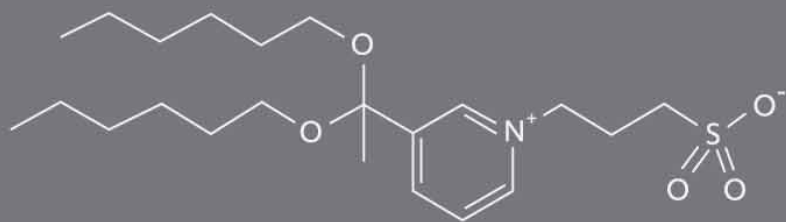
- Permits broad mass range analyte fractionation, targeting specific MW
- Analytes are recovered intact for characterization of PTMs, truncations, alterations, immunoaffinity
- High protein recovery across the entire mass range
- Fractions are easy to harvest with a pipette (no slicing of gel bands)
- Permits in-solution digestion vs. in gel-digestion
- High reproducibility (RSD <5%), high loading capacity (>200µg per channel)
- Non biased sampling of protein classes (hydrophobic, acidic, basic, LMW, etc.)
- 8 channels in 90 minutes

PPS Silent[®] Surfactant

- MS-Compatible Detergent for Solubilizing Membrane Proteins -

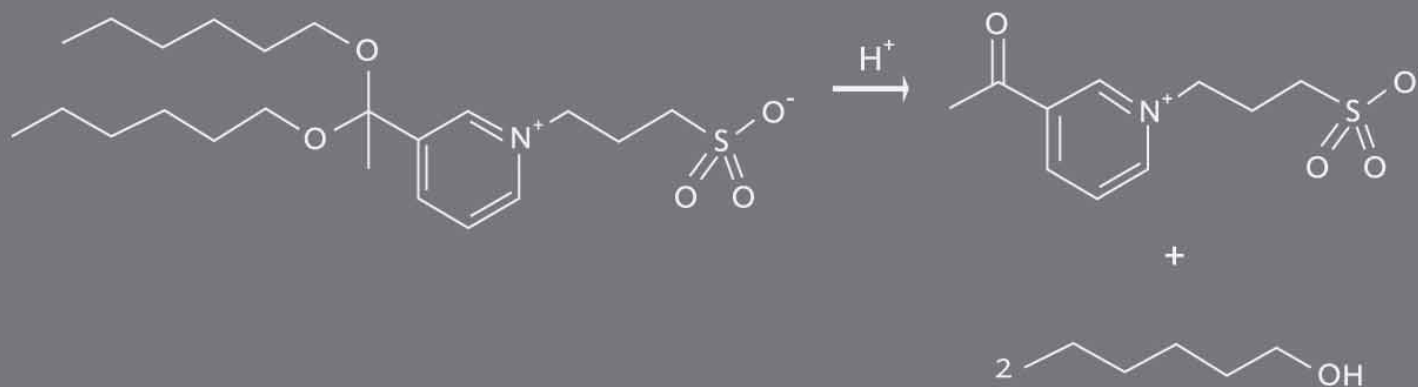
THE SURFACTANT

Intact PPS Silent Surfactant disrupts cell membranes and solubilizes hydrophobic proteins.



THE CLEAVAGE

Add acid to reduce the pH and cleave PPS.
The reaction products are soluble and have no surfactant properties.



MEMBRANE PROTEINS

"We were astonished to find that greater than half of the proteins identified are classified as membrane proteins"

Table 1. Proteins Identified by nanoLC-MS/MS

SAMPLE ID	PROTEINS IDENTIFIED	PEPTIDES IDENTIFIED
Patient 24	985	1972
Patient 26	844	1736

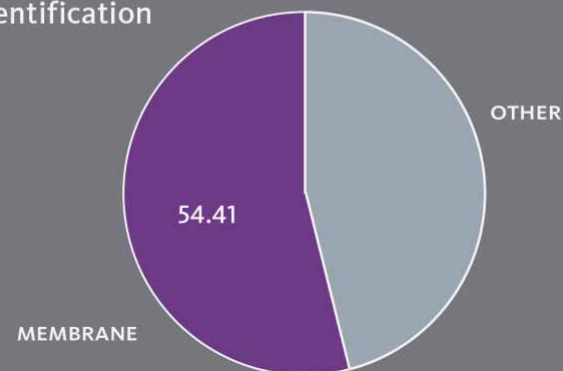
Table 2. Proteins Identified ≥ 2 peptides by nanoLC-MS/MS

SAMPLE ID	PROTEINS IDENTIFIED
Patient 24	620
Patient 26	468

Single-tube
Protocol



Protein
Identification



ADVANTAGES

- Improves MS analysis of complex protein mixtures
- Disrupts cell membranes
- Solubilizes hydrophobic proteins
- Improves enzymatic digestion
- Cleaves readily at low pH
- Cleavage products have no detergent properties and stay in solution for easy removal

INNOVATIONS IN SAMPLE PREP

