

3000 Series Gradient Formers

User Guide

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For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.



WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

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Considerations

This document uses the following conditions:



DANGER Indicates a hazardous situation which, if not avoided, will result in death or serious injury.

WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE Follow instructions with this label to avoid damaging the system hardware or losing data.

Note Contains helpful supplementary information.

Site Preparation and Safety

Before using the system, read the site preparation and safety manual on the documentation media provided. Always follow the safety precautions in that manual and in this document when using the system.

Operating Precautions



WARNING Do not operate this system without following the safety precautions described in this manual and the documentation that came with your system.

The gradient former contains precise electronic components. Handle it carefully and follow these precautions.

- Do not allow moisture to leak into the instrument interior
- Wipe off spilled chemicals immediately
- Do not drop the instrument
- Protect the instrument from mechanical shock
- Protect the instrument from dust

Sucrose Gradient Background

Ripped unabashedly from *Molecular Biology of the Cell*, 4th edition. “Although biochemical analysis requires disruption of the anatomy of the cell, gentle fractionation techniques have been devised to separate the various cell components while preserving their individual functions. Just as a tissue can be separated into its living constituent cell types, so the cell can be separated into its functioning organelles and macromolecules.

Cells can be broken up in various ways: they can be subjected to osmotic shock or ultrasonic vibration, forced through a small orifice, or ground up in a blender. These procedures break many of the membranes of the cell (including the plasma membrane and membranes of the endoplasmic reticulum) into fragments that immediately reseal to form small closed vesicles. If carefully applied, however, the disruption procedures leave organelles such as nuclei, mitochondria, the Golgi apparatus, lysosomes, and peroxisomes largely intact. The suspension of cells is thereby reduced to a thick slurry (called a *homogenate* or *extract*) that contains a variety of membrane-enclosed organelles, each with a distinctive size, charge, and density. Provided that the homogenization medium has been carefully chosen (by trial and error for each organelle), the various components—including the vesicles derived from the endoplasmic reticulum, called microsomes—retain most of their original biochemical properties.

The different components of the homogenate must then be separated. Such cell fractionations became possible only after the commercial development in the early 1940s of an instrument known as the *preparative ultracentrifuge*, in which extracts of broken cells are rotated at high speeds. This treatment separates cell components by size and density: in general, the largest units experience the largest centrifugal force and move the most rapidly. At relatively low speed, large components such as nuclei sediment to form a pellet at the bottom of the centrifuge tube; at slightly higher speed, a pellet of mitochondria is deposited; and at even higher speeds and with longer periods of centrifugation, first the small closed vesicles and then the ribosomes can be collected. All of these fractions are impure, but many of the contaminants can be removed by resuspending the pellet and repeating the centrifugation procedure several times.

When a homogenate is sedimented through dilute sucrose gradients, different cell components separate into distinct bands that can be collected individually. The relative rate at which each component sediments depends primarily on its size and shape—being normally described in terms of its sedimentation coefficient, or *s* value. Present-day ultracentrifuges rotate at speeds of up to 80,000 rpm and produce forces as high as 500,000 times gravity. With these enormous forces, even small macromolecules, such as tRNA molecules and simple enzymes, can be driven to sediment at an appreciable rate and so can be separated from one another by size. Measurements of sedimentation coefficients are routinely used to help in determining the size and subunit composition of the organized assemblies of macromolecules found in cells.” See Figure 1 for a visualization.

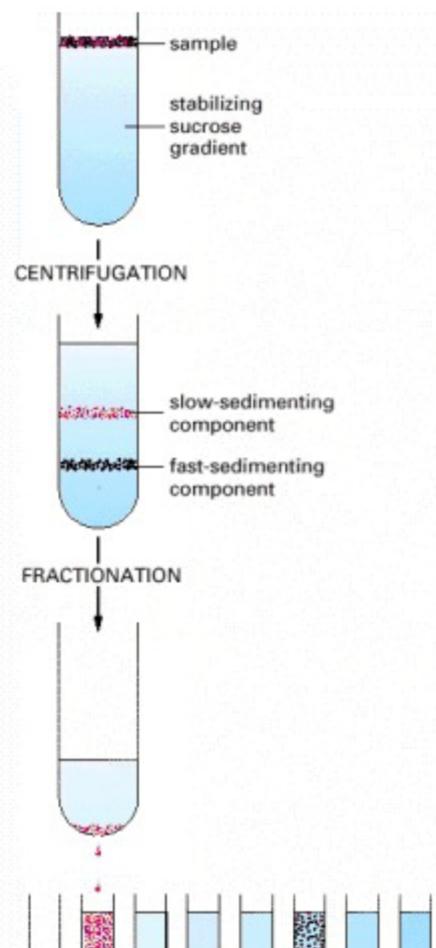


Figure 1: Dilute Sucrose Sedimentation.

Common Use Cases for Sucrose Gradient Sedimentation

- Analysis of cellular translation state via polyribosome profiling

Polyribosome profiling relies on in-line detection of rRNA during fractionation, allowing users to record chromatograms of absorbance peaks that correspond to different ribosomal species. Lighter fractions contain smaller species (i.e. monosome/disome) and heavier fractions contain polyribosomes. The degree of translation is quantified by integrating absorbance peaks of polysome-containing fractions. In this example, upon ER stress, absorbance increases in monosome fractions and decreases in polysome fractions relative to a control sample, so it is logical to conclude that ER stress results in a global decrease in translation.

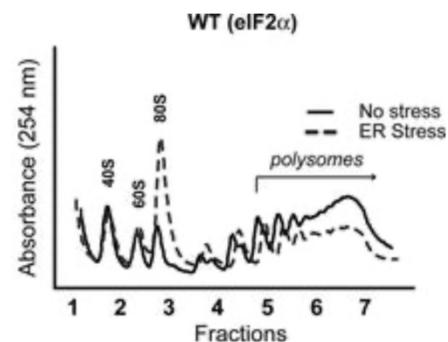


Figure 2: Baird et al. 2014, PMID: 24648495

- Analysis of protein oligomerization

As proteins oligomerize, they become heavier and travel faster through a sucrose gradient. IRE1 oligomerizes during protein folding stress. Gradients are fractionated and IRE1 is detected by western blot. Increased or more stable oligomerization is evidenced by IRE1 populating heavier fractions relative to a control sample.



Figure 3: Takeda et al. 2019, PMID: 31368599

- Purification of organelles

Organelles vary in density and Svedberg values, which can be used to separate and purify them from sucrose gradients.

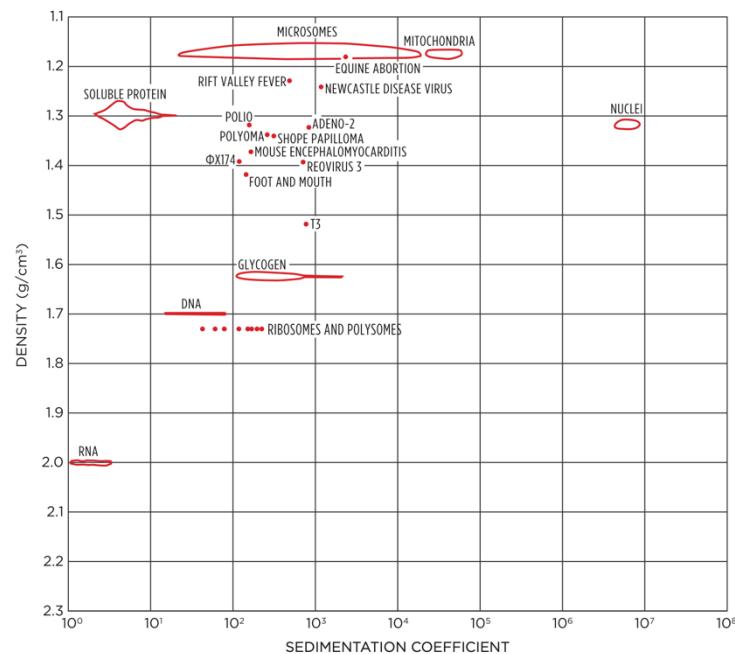


Figure 4:
https://ls.beckmancoult.co.jp/files/cases/Fundamentals_of_Ultracentrifugal_Virus_Purification.pdf

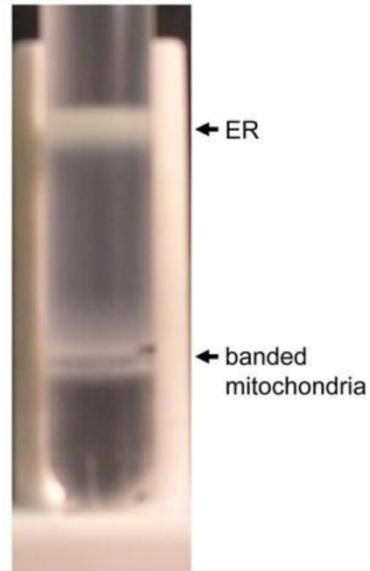


Figure 5: Williamson et al. 2016, PMID: 26331984

Conventional Methods for Sucrose Gradient Formation

Most protocols will require layering multiple concentrations of sucrose and subjecting the gradients to freeze-thaw cycles to encourage diffusion among layers. Others will have you operating erratic peristaltic pumps to mix sucrose solutions for deposition in an ultracentrifuge tube.

These methods have three obvious disadvantages:

- 1) Not only is layering multiple sucrose solutions tedious, pipetting error associated with multiple layering events accumulates. In the case where you make six gradients, they will rarely be identical, leading to reproducibility issues.
- 2) Freeze-thaw cycles take up to two days following common protocols – two days later than you want your result.
- 3) Both methods rarely produce continuous gradients. This reduces resolution, leading to inefficient separation of macromolecules.

Superior Method for Sucrose Gradient Formation

David Coombs founded BioComp and subsequently produced The Gradient MasterTM, the use of which to produce a sucrose gradient formation requires only two sucrose solutions, minimizing pipetting error. To enhance reproducibility, six perfectly linear gradients are simultaneously formed. All this in less than two minutes because your time is valuable, and he appreciates you.

The Gradient MasterTM generates linear gradients using BioComp's patented process called "tilted tube rotation." The process involves layering the two end point solutions (i.e. 5% and 30% sucrose) directly in the centrifuge tube, capping it and placing it in a magnetic tube holder (MagnaBaseTM) that adheres to the rotary steel plate on the instrument. The Gradient Master takes over and tilts the tube to the proper angle, rotates it for a designated time, and returns it to its original vertical position, ready to use. The only problem? This things is priced at \$6000 USD.

We, at Dumpster Baby Industries, have liberated this technology for the good of all – for the graduate students tired of that sinking feeling associated with gradient formation – for the PI tired of being nickel and dimed for everything with the word "scientific" attached – for all the fun it will be to face the legal ramifications if David ever finds this document.

Basics of the Dumpster Baby Industries 3000 Series™ Gradient Former

The DBI 3000 Series™ has everything you need and nothing you don't. An integrated power switch emboldens you to leave this gradient-former plugged in, sans fear of roasting the microchip brain. Robust red buttons allow you to burst onto the scene and forcefully make a selection. A state-of-the-art 20x4 LED screen displays the numerous gradient offerings. The SW-41 tube rack is compatible with Beckman and Seton tubes (UltraClear and polyallomer). Two of the most precise 1.8-degree stepper motors produced are mounted to mix your hopes and dreams into a linear solution. All encased in a pristine Bio-Rad power supply – a relic worthy of saving from the dumpster.

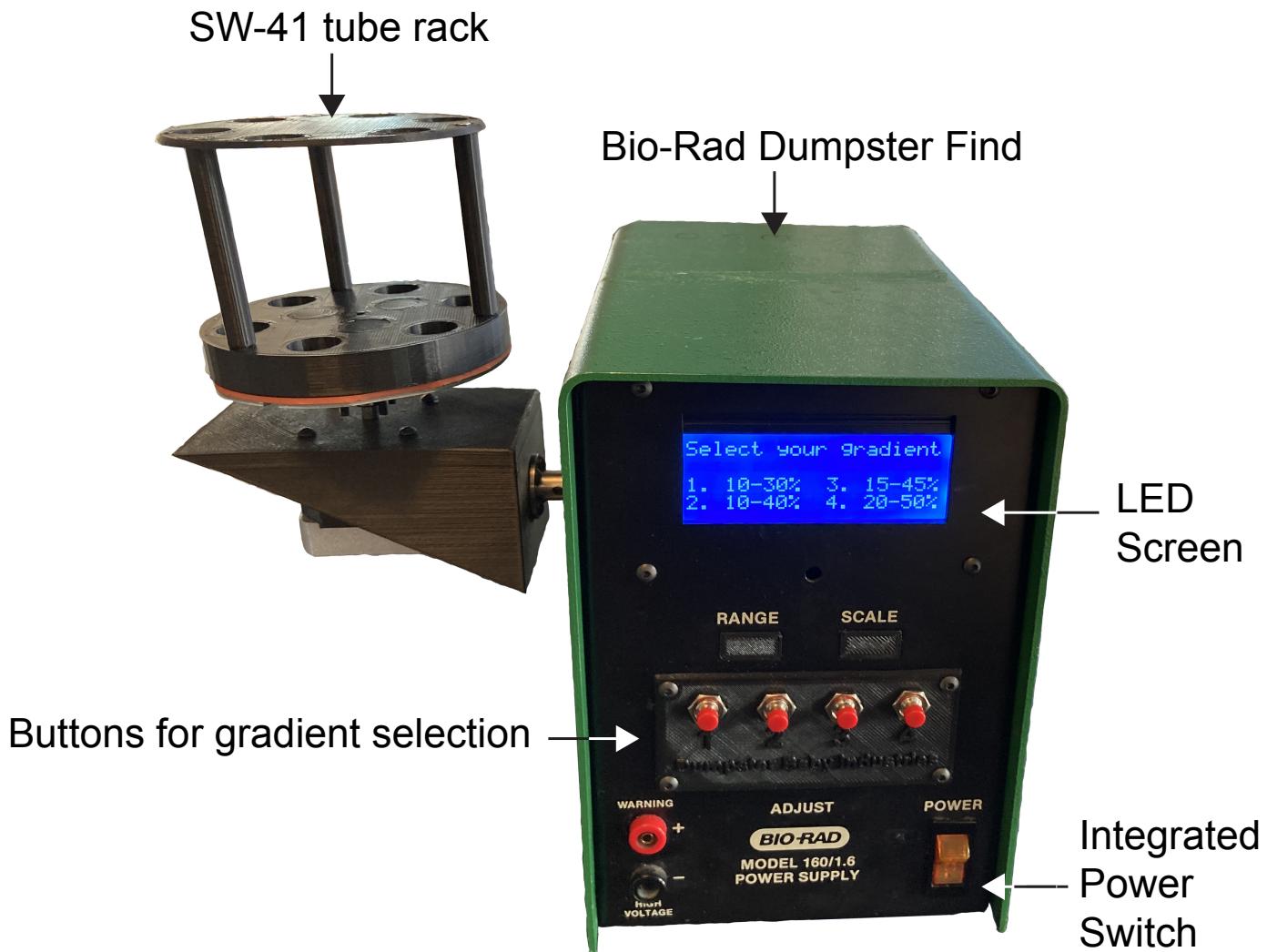


Figure 6: Anatomy of the DBI 3000 Series™ Gradient Former

Practical Use – Dumpster Baby Industries 3000 Series™ Gradient Former

Every gradient starts with making sucrose solutions. Choose from the following sucrose gradient offerings:

1. 10-30%
2. 10-40%
3. 15-45%
4. 20-50%

If you desire additional gradient selections, consult technical support at the address listed on page 2 for a quote or to schedule a visit from one of our certified technicians.

Basic Workflow

1. Layer the densest sucrose solution below the lighter solution and set them in a stable rack.
2. Cap the tubes with the Dumpster Baby Industries reproduction of David's patented caps.
3. Ensure the DBI 3000 Series™ is plugged into the wall.
4. Gently set the magnetic SW41 tube rack onto the rotisserie base.
5. Calibrate the base angle with a level and use the integrated power switch to turn the DBI 3000 Series™ ON.
6. Now that the rack is level and locked in place, load your tubes into the rack.

IMPORTANT: The machine must be powered for the rotisserie platform to lock in place. If you fail to perform these steps in order, your sucrose solutions will move spin straight to the bench and spill.

7. After loading your tubes in the rack, press the numbered button corresponding to the gradient you wish to form.
8. Sit back and wait for the tubes to spin and return to their original position – you have produced linear sucrose gradients, yay!
9. Remove your tubes from the rack, then turn off the DBI 3000 Series™ via the integrated power switch.

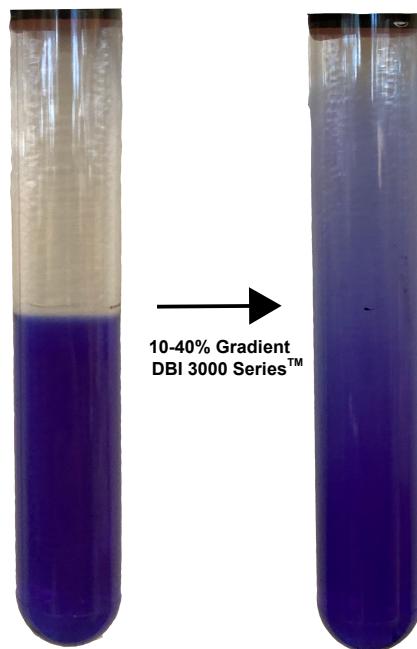


Figure 7: Result of using the superior gradient formation technique.

