Lab Manager®


# Eppendorf Solutions for High-Speed and Ultracentrifugation

**Resource Guide**

- What to Consider When Choosing an Ultracentrifuge for Your Lab
- Maximizing Ultracentrifuge Safety and Convenience
- Unique 4 x 1.5L Capacity Rotor for High-Speed Centrifuges CR22N and CR30NX
- Density Gradient Centrifugation Applications and Throughput
- Isolation and Enrichment of Golgi Bodies from Rice Seedlings Using Density Gradient Ultracentrifugation

Lab Manager

High-speed and ultracentrifuges, running at speeds from 50,000 to 1,000,000 times gravity, are requisite instruments for many labs, enabling finer resolution separations for improved purity. They require additional considerations around size, throughput, and safety in determining which instrument is the best fit for your lab-space and workflows.

This eBook takes a closer look at each area of consideration along with applications for Eppendorf's new high-speed floor-standing centrifuge and ultracentrifuge series. Particular focus is given to considerations for choosing ultracentrifuges, safety features and best practices, and how a single floor-standing centrifuge can pivot from high-volume harvesting protocols to sensitive density gradient centrifugation protocols.

# What to Consider When Choosing an Ultracentrifuge for Your Lab

**With so many highly specialized options available, care must be taken when selecting the right ultracentrifuge for your lab**

by Erica Tennenhouse, PhD, Rachel Brown, MSc, and Eppendorf

Ultracentrifugation accomplishes faster separations and higher resolution of similarly sized particles than either standard or high-performance centrifugation. This ultrafast technology is ideally suited to a variety of exciting research applications. But with so many highly specialized options available, care must be taken when selecting the right ultracentrifuge for your lab.

## MANY USES

Preparative ultracentrifugation, which covers most applications, is used to separate and purify small particles for some downstream processes. Applications requiring preparative ultracentrifugation are many and varied, from lipoprotein separation to nucleic acid purification and subcellular fractionation. Advancements have enabled virus particle separation and purification, with broad applications in vaccine production. Ultracentrifuges are also preferred for exosome purifications, another popular application. These vesicles were once thought to simply contain junk from the cell, but scientists now understand that exosomes are involved in cellular communication, which makes them far more interesting from a research perspective.

## THINK BEFORE YOU SPIN

When sifting through all of the ultracentrifuge options available, users would be wise to consider several factors, including rotational speed, rotor type, size, and consumables.

The sizes and sedimentation rates of particles being separated will dictate the speed required. The smaller the particles requiring separation are, the higher the g forces required.

There are several types of rotors that can be used for ultracentrifugation, including fixed-angle, neo angle, vertical, and swing-bucket to suit any application. While fixed-angle, neo angle, and vertical rotors hold tubes in a fixed position relative to the central axis, swing-bucket rotors allow tubes to swing outward while they spin.

Ultracentrifuge models come in different sizes. Though larger floor models are common, a compact benchtop design might be a better choice for labs that are limited on space.

It is important to think about the consumables available as well. Users should consider both how a particular tube will enable them to retrieve their sample and how well the tube is able to withstand high centrifugal forces.

## ULTRA-ADVANCED TECHNOLOGY

Although ultracentrifugation has been a widely used technology for some time, there have been recent innovations. For instance, the Eppendorf CP-NX line of ultracentrifuges track cumulative run time at every speed—including during ramping stages—for each rotor. This offers the most accurate and precise logs possible, allowing users to get the most use out of each rotor's lifespan safely.

Due to the fact that a centrifuge of any speed is often a shared resource, scheduling software is frequently used to control and monitor centrifuges remotely. With such software, users can schedule their time with the centrifuge and guarantee that no one else jumps the queue. Some instruments, like the CP-NX line, even offer individual logins for differing levels of user access to ensure usage is commensurate with training.

# Eppendorf Ultracentrifuges and Micro-Ultracentrifuges

When ultra-speeds are required, the refrigerated Ultracentrifuge CP-NX Series and Micro-Ultracentrifuge CS-NX Series maximize safety and efficiency while providing high-quality sample preparation in a user-friendly design.

- Extraordinarily high speeds of up to 803,000 x g (CP-NX Series) or 1,050,000 x g (CS-NX Series) plus fast acceleration and deceleration ramps ensure speedy separation of samples such as nucleic acids, proteins, vesicles, viruses and more.
- Automatic Rotor Life Management System (CP-NX Series) significantly increases rotor lifetime by automatically tracking the exact run times on the internal rotor memory, making handwritten rotor logs a thing of the past.
- The self-locking rotor system eliminates the need for tools or manual tightening, enabling quick and easy rotor exchange in seconds.
- The innovative, non-contact imbalance sensor ensures safe operation, while the possibility to balance samples by eye (up to 5 mm*) reduces time-consuming sample balancing via scale.
- The intuitive touchscreen user interface with fast start-up provides enhanced security and compliance with multi-level, user-based access control. Programming and documentation of runs supports multi-user labs as well as laboratories working in GxP/GLP compliance.


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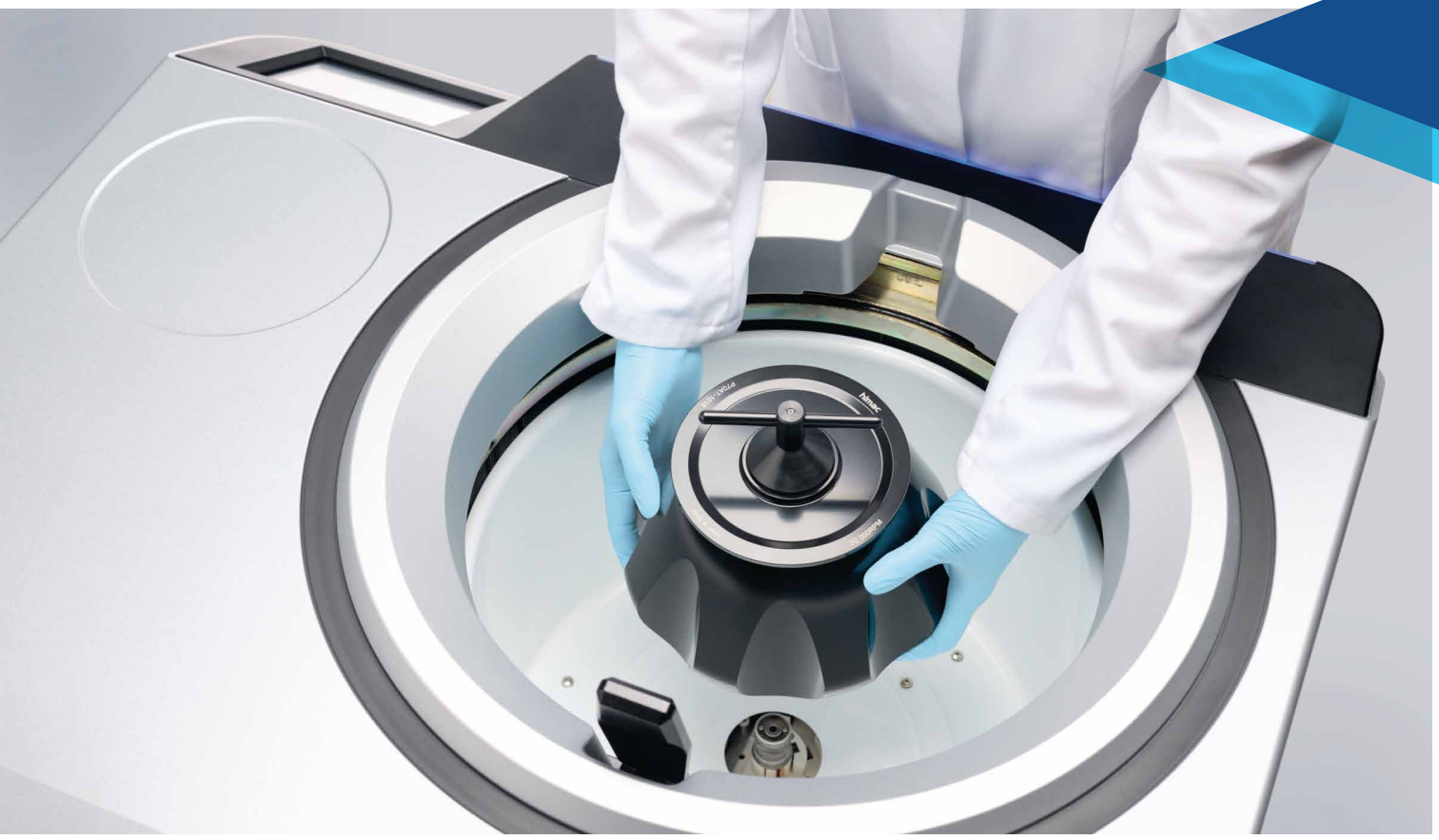
**Except for rotors P21A2, S140AT, S110AT, S80AT3, S50A*



# Maximizing Ultracentrifuge Safety and Convenience

## Instrument features, best practices, and maintenance instill confidence in the safe use of ultracentrifuges

by Eppendorf

Despite forces surpassing 1,000,000 times gravity, safe operation of ultracentrifuges can be ensured by following best practices and regular maintenance checks. As with most lab equipment, proper training and maintenance combined with modern safety features are key to protecting samples, equipment, and staff, while service agreements can contribute peace of mind.

### STAY IN BALANCE

Ultracentrifuges require additional care and attention to balancing loads above general centrifuge standards. The increased force makes them more sensitive to imbalances—relatively small differences in mass between samples can have a large impact. Instruments in Eppendorf's CP-NX ultracentrifuge series solve this problem neatly with a highly precise imbalance detection utilizing a non-contact imbalance sensor, which allows balancing by sight rather than scale.* For most instruments, however, opposing tubes should be balanced by weight using a precise lab scale to within the manufacturer's specifications. Where additional tubes are required for balance, use liquids of a similar density to the samples, ensuring tubes are balanced by mass. Whereas a three-point balance is suitable for standard centrifuges, every tube should have a balanced tube in the opposite position for ultracentrifuges.

Swing-bucket rotors come with a few more considerations. They must only ever be used with all buckets in place. Users should never exceed a bucket's weight limit. Each pivot should be checked prior to use and cleaned or lubricated as necessary to ensure they swing smoothly. Load buckets with tubes arranged symmetrically and centrally, applying the load equally to each pivot.

### LOOK TO THE ROTORS

Every rotor has a maximum speed or g-force limit. Many modern instruments have automatic rotor detection coupled with overspeed detection to ensure these limits are never exceeded, but if not, users must take care to follow each rotor's guidelines.

Rotors also have defined lifespans based on their type and materials. Ideally, usage should be tracked for each rotor and the rotor replaced at the end of its life. Asset management software can help with this potentially tedious task, though it will likely take

*Except for rotors P21A2, S140AT, S110AT, S80AT3, S50A

manual intervention to track specific rotors on the same instrument. The CP-NX series goes a step further to log individual rotor use on-board with precise cumulative run time at every speed so users can safely maximize usage of individual rotors. Rotor lifespans may be reduced by misuse or stress from imbalanced loads, so regular visual checks are still needed.

Always ensure rotors are in good condition and fully attached before use. Look to self-locking rotors like those in the CP-NX series to make the process of changing rotors easier and safer. Good condition can easily be maintained by following best practices. Clean rotors and drive shaft weekly or monthly depending on use, including rotor lid, rubber seal, buckets, and adapters, using a damp lint-free cloth and diluted detergents or alcohol. Ultracentrifuges are refrigerated and so should be wiped down after each use to remove condensation, the chamber should be defrosted as necessary, and the water collection tray emptied and cleaned.

## CONFIDENCE-BOOSTING SERVICE

A good service plan frees more time and resources for research. Eppendorf offers flexible full-service plans including GxP installation and operational qualifications. Other peace-of-mind options to look for when shopping service plans include installation and commissioning as well as full annual preventive maintenance service.

Use, care, and maintenance of ultracentrifuges can be made easier with the right models and service. Safety and convenience can be further improved with the thoughtful additional features provided in Eppendorf's CP-NX ultracentrifuge series. Look for a balanced array of safety features and service options to safeguard both lab staff and equipment.

# Unique 4 x 1.5 L Capacity Rotor for High-Speed Centrifuges CR22N and CR30NX

by Aurélie Tacheny

## EXECUTIVE SUMMARY

Equipped with a broad collection of different fixed-angle and swing-bucket rotors, the high-speed Centrifuges CR22N and CR30NX are perfectly fitted for a wide range of applications, ranging from biomass harvesting to higher speed pelleting applications (up to 110,000 times gravity in Centrifuge CR30NX with Rotor R25ST and Rotor R30AT).

One of the most common applications for these types of centrifuges is the harvesting of biomass, such as bacteria, yeast, or cell cultures. Processing batch volumes of several liters requires successive time-consuming steps to be repeated with every vessel to be centrifuged. It is now possible to reduce the number of bottles that are needed from six to four bottles with Rotor R9A2—the unique rotor design greatly improves the harvesting process efficiency by reducing the number of vessels needed with a similar centrifugation capacity and consequently the process time (32 percent time savings). Processing up to four bottles of 1.5 L (6 liters) at up to 15,100 times gravity, this fixed-angle rotor is ideal for the harvesting of bacteria, mammalian or insect cells, algae, or yeast. Moreover, the unique triangular wide-mouth bottles 1.5 L are specifically designed to improve process flexibility by allowing users to process any initial batch volumes, since no filling volume restriction is imposed in contrast to commonly used large volume centrifugation vessels.

## INTRODUCTION

Biomass harvesting is the initial downstream process step of every workflow related to bioprocess, such as DNA plasmid preparation or recombinant protein purification. As soon as several liters of cell culture need to be handled, floor-standing centrifuges in combination with large capacity rotors are commonly used, sometimes requiring successive runs to process the complete batch volume. Although its principle is relatively simple, this preliminary harvesting step consists of a succession of small, time-consuming steps to be repeated with every vessel to be centrifuged, starting from bottle filling, balancing, tight bottle closure and rotor loading to supernatant decanting, pellet recovery, and finally bottle washing and autoclaving (as illustrated below). Consequently, every feature that simplifies this process will greatly improve workflow efficiency.

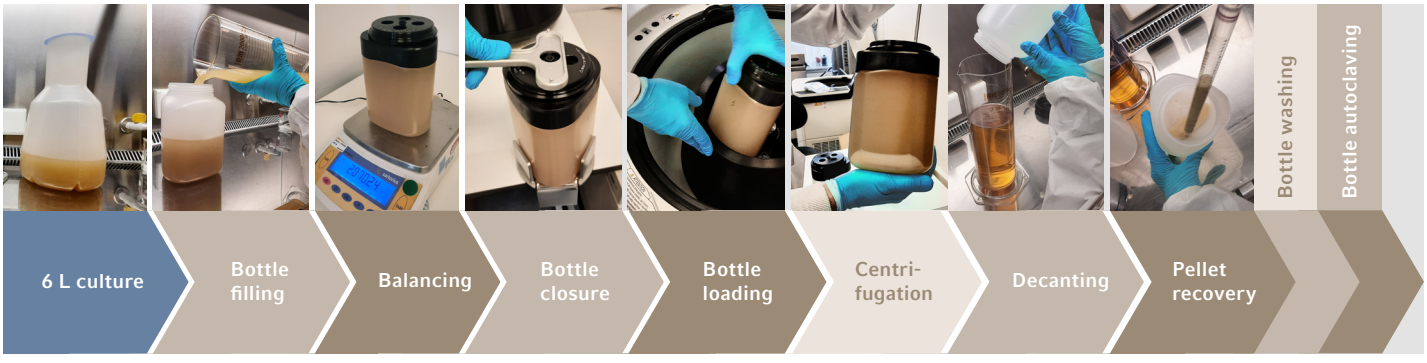



**Figure 1**: Handling steps in cell culture harvesting

## SOLUTIONS & BENEFITS

Presenting a unique and compact design, the R9A2 fixed-angle rotor allows users to process four bottles of 1.5 L per run, with a total capacity of six liters. Suitable for both high-speed Centrifuges CR22N and CR30NX, this rotor is perfectly suited for harvesting of bacteria, algae, yeast, mammalian, or insect cell cultures with a maximum speed of 15,100 times gravity. In contrast to commonly used large capacity fixed-angle rotors, this rotor reduces the number of vessels to be handled by researchers from six to four with a similar centrifugation capacity. Thanks to this unique feature, process efficiency can be greatly improved without impacting the pelleting efficacy. As illustrated in the table below, precise labor time assessments of every successive step through two parallel bacteria harvesting workflows showed that this solution saves 19 minutes in comparison with a conventional 6x1L capacity rotor, corresponding to 32 percent of the complete processing time.

**Figure 2**: Unique triangular 1.5 L bottle

| Rotor R9A2<br>4x1.5 L bottles | | Competitor<br>6x1 L bottles |
|---|---|---|
| | 6 L culture | |
| ~06:30 | Bottle filling | ~09:30 |
| ~01:00 | Balancing | ~03:00 |
| | Bottle closure | |
| | Rotor loading | |
| | Centrifugation | |
| ~05:00 | Decanting | ~08:00 |
| ~11:00 | Pellet recovery | ~17:00 |
| ~14:30 | Bottle washing | ~17:30 |
| | Bottle autoclaving | |
| **~37:00** | **Processing time (min)** | **~55:00** |

**Figure 3:** Handling time comparison four vs. six bottles

Made of PPCO (polypropylene copolymer), the 1.5 L bottles present excellent chemical and mechanical resistance. Together with their unique triangular shape, a wide-mouth opening, and the tight closure system, they are specifically designed for harvesting applications. Their exclusive design is intended to improve process flexibility by allowing users to process any initial batch volume, since no minimum filling volume is needed. In this respect, the triangular bottle 1.5 L as well as 1 L, 500 mL, and 250 mL flat-bottom bottles stand out strongly from conventional volume centrifugation vessels*. As long as they are balanced, these bottles can be centrifuged with any volume from 0 to their respective maximum capacity volume.

*Competitor centrifuge bottles require a minimum filling volume of 80 percent due to limited mechanical resistance.

For more information, please check our local website or contact your local sales representative.

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# Eppendorf High-Speed, Floor-Standing Centrifuges

From harvesting up to 6 L of biomass per run to separation steps of small particles such as viruses, organelles, and vesicles, the high-speed refrigerated Centrifuge CR22N and Centrifuge CR30NX offer the performance and versatility needed for a wide range of applications.

- Speeds up to 58,700 x g (CR22N) support applications in genomics, cell biology, and proteomics while speeds up to 110,000 x g (CR30NX) enable super-speed applications such as density gradient centrifugation and cell fractionation.
- Save time with the unique 1.5 L triangular bottle, which provides easy and efficient harvesting of cells, yeast, and bacterial cultures in volumes up to 6 L per run.
- Automatic rotor identification and the self-locking rotor system enhance safety while making rotor exchange quick and convenient.
- The high-resolution, color LCD touch interface at the front of the centrifuge features a clean, intuitive layout for fast and easy operation.
- User management with two user access levels plus programming and documentation of runs support labs working in a GLP/GxP environment.


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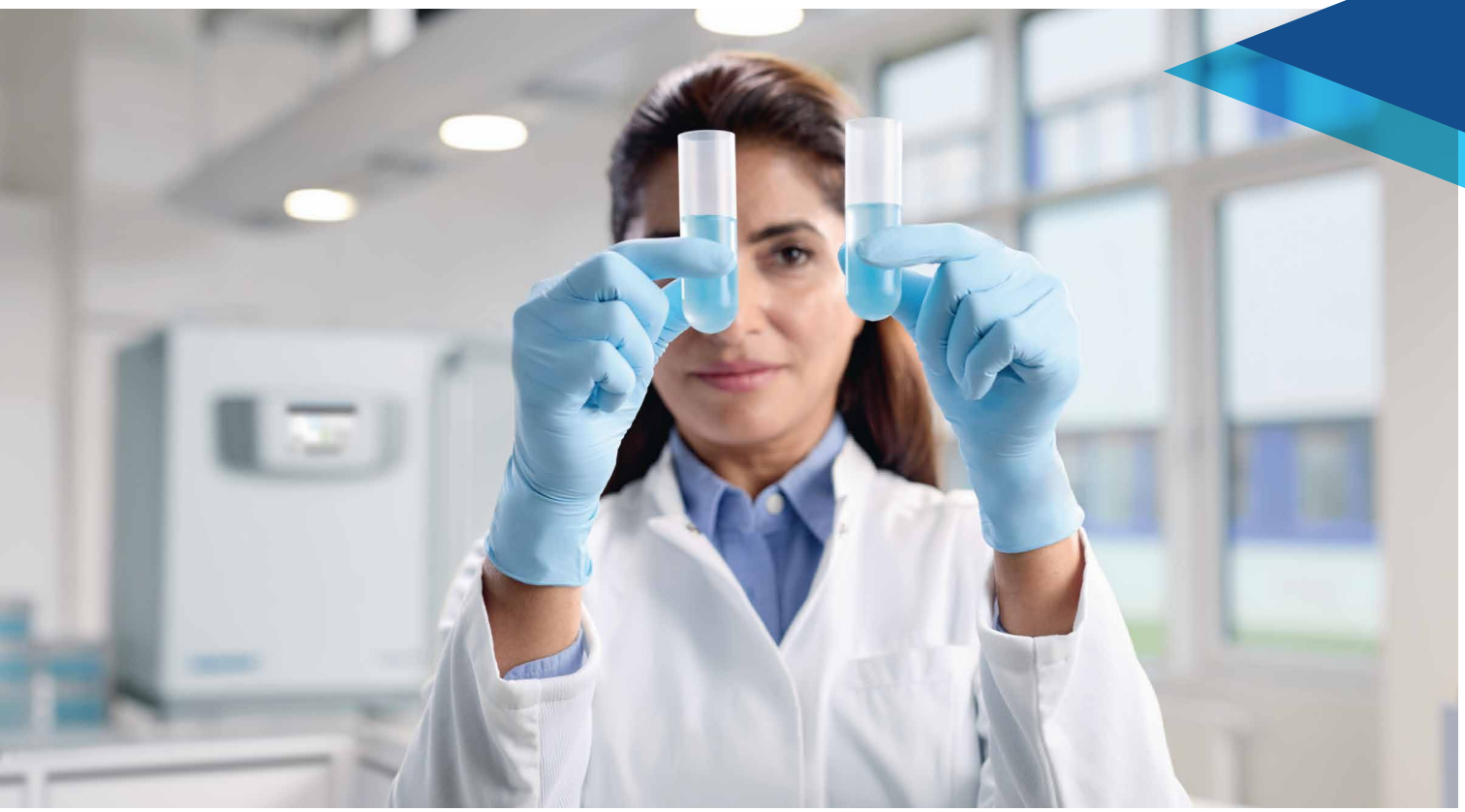

# Density Gradient Centrifugation—Applications and Throughput

**Density gradient centrifugation, the gold standard for purification of biomolecules and structures, is increasing in accessibility and throughput**

by Eppendorf

Density gradient ultracentrifugation is widely considered the gold standard for purification of extracellular vesicles, viral particles, and other biomolecules and small structures for both its efficiency and purity. Its primary limitations center on accessibility and throughput, specifically around ultracentrifuge cost and process volume. Though the finest resolutions require an ultracentrifuge operating at forces up to 1,000,000 times gravity, separations are often run at lower speeds equating to 100,000-200,000 times gravity, within the range of higher volume high-speed centrifuges.

## WHAT IS DENSITY GRADIENT CENTRIFUGATION?

Preparative centrifugation is a standard means of isolating and purifying macromolecules or nanoparticles of interest from a solution, commonly applied to separation and purification of cellular components like organelles or vesicles, DNA, and viruses.

Separation via preparative centrifugation is divided into two types of methodology—differential centrifugation and density gradient centrifugation. Differential centrifugation, consisting of one or more pelleting steps, separates components sequentially based on their respective sedimentation rates. This method is good for coarse separations, where there is a large difference between components.

Density gradient separation uses a solution density gradient formed by adding layers to the tube in increasing density from top to bottom or by spinning a solution until a solution gradient forms. The sample is added to the solution, usually salt- or sucrose-based, spun, and distributed in layers based on density or sedimentation rate. This method allows distinct layers of interest to be drawn from the tube, offering greater resolution of smaller components, and is excellent for separating components with similar molecular weights but different densities.

Density gradient separations can be performed using two different techniques—by differential sedimentation rate (rate-zonal) or by particle alignment to the distinct density layers (isopycnic).

Rate-zonal separation is time-dependent. Components move through the solution according to their respective sedimentation rates but are denser than the solution and will all eventually pellet. The spin is stopped while all components are still suspended in layers.

Isopycnic separations reach equilibrium with components aligned to layers that match their buoyant density. This separation is stable through time and will be maintained with continued spinning.

## COMMON SOLUTIONS AND APPLICATIONS

Sucrose density gradient centrifugation is widely applied to separation of cellular components and biomolecules using both

rate-zonal and isopycnic strategies. Sucrose isopycnic centrifugation is recommended for examining immune complexes, however sucrose binds to lipoprotein complexes and must be used with caution for related isolations.

Saline solutions including NaCl, NaBr, and/or KBr salts are commonly applied to lipoprotein analysis, and cesium chloride density gradient centrifugation is used to separate and purify DNA molecules. Cesium chloride forms a density gradient from the heavy dissolved cesium ions via centrifugation, and offers very fine resolution, for example, separating DNA formed with δ4N from δ5N.

Density gradient centrifugation is well-suited to some difficult purifications. For example, it can resolve tagged structures from dye aggregates while removing free fluorescent dye, outperforming size-based purification options. Isopycnic separation can resolve microsomes from viruses, despite overlap in their sedimentation rates.

Throughput is the primary limitation for gradient centrifugation protocols, though advancements in high-speed floor-standing centrifuges improve capacity. For example, the Eppendorf Centrifuge CR30NX can accommodate six 40 ml tubes at 110,000 times gravity, sufficient to separate distinct classes of small particles. Such multipurpose centrifuges allow users to move from cell fractionation to density gradient separation without switching instruments, which also increases the accessibility of these techniques.

# Isolation and Enrichment of Golgi Bodies from Rice Seedlings Using Density Gradient Ultracentrifugation

by Kazusato Oikawa, Shuichi Kani, and Mark Hünken

## ABSTRACT

Himac, now part of the Eppendorf Group, has a profound record of over 60 years of experience developing high-speed floor-standing ultracentrifuges tailored to customer needs. In this application note, the use of the CPNX-series ultracentrifuge (here CP80NX) in combination with swing-bucket rotor P32ST and rotor P40ST from Himac for the isolation of Golgi from rice seedlings will be demonstrated. Obtaining high-quality Golgi isolates is critical for further analysis and understanding of this organelle. It is shown how this can be achieved by the sequential application of differential pelleting and discontinuous sucrose density gradient centrifugation.


**Figure 1:** Ultracentrifuge of the CP-series in combination with swing-bucket rotors P32ST and P40ST

## INTRODUCTION

The Golgi apparatus of eukaryotic cells was first described more than 120 years ago by Camillo Golgi. Advances in (electron) microscopy revealed the complex structure while further biochemical analysis exposed various functions of this organelle within the cell [2].

In cells of higher organisms, the Golgi apparatus is responsible for the synthesis of complex polysaccharides and the processing and distribution of proteins to other organelles as part of the secretory pathway (Figure 2) [1].

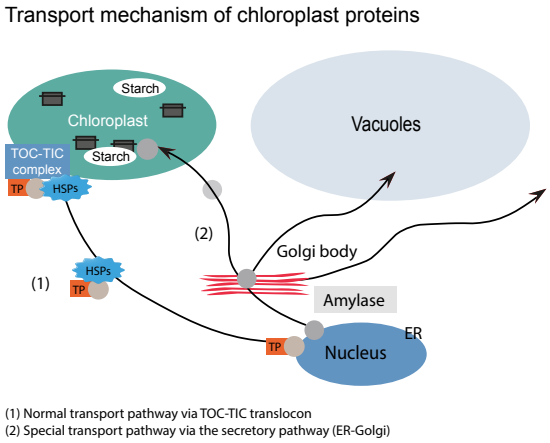


**Figure 2:** Protein transport in plant cells via (1) the Toc-Tic translocatio and (2) via the Golgi apparatus and the secretory pathway.

One example of such a protein is δamylase, a glycosidase responsible for the hydrolysis of starch molecules within plants. It was shown that δamylase is synthesized at the endoplasmic reticulum (ER) ribosomes, glycosylated within the ER-lumen, and then transported into the Golgi apparatus for oligosaccharide modification [3]. However, as the Golgi apparatus forms a complex structure with other membrane systems like the endoplasmic reticulum (ER) [2], it is particularly difficult to isolate distinct parts of this organelle. Indeed, fractions of Golgi membranes are often contaminated with parts of other connected membrane systems like vacuoles [2]. Density gradient centrifugation is one of the most established techniques used for the enrichment of specific membranes [2]. In this application note, we describe a technique using a sequence of differential pelleting and density gradient centrifugation to obtain fractions of Golgi apparatus membranes from rice seedlings. The applied technique provides extracts of high purity and quality for further downstream analysis like mass spectrometry. The isolation of high-quality Golgi fractions using Centrifuge CP80-NX with the combination of the rotors P32ST and P40ST will be described in the following section.

## MATERIALS AND METHODS

*Materials used*

Centrifuge CP80NX with the following swing-bucket rotors:
- Rotor P32ST for 40 mL PET tubes
- Rotor P40ST for 13 mL PET tubes

*First step:*

Microsome purification process using swing-bucket rotors P32ST (40 mL PET tube) and P40ST (13mL PET tube).

1. Centrifuge the purified-rice extract at 15,000 × g for 30 min at 4 °C in 40 mL PET tubes in a swing-bucket rotor and discard the pellet.
2. Load the 11 mL of supernatant on top of 1 mL of 15% sucrose solution over 1 mL of 50% sucrose cushion in 13 mL PET tubes.
3. Centrifuge at 100,000 × g for 3 h at 4 °C and subsequently collect the microsome fraction trapped on the cushion of 50% sucrose solution.

*Second step:*

Golgi purification process from microsome fraction by using swing-bucket rotor P40ST (13 mL PET tube).

1. Adjust the collected fraction to 42% sucrose density with 60% sucrose buffer using a refractometer. Load 1-2 mL of this solution to another discontinuous sucrose density gradient consisting of 1 mL layers of 26%, 30%, 34% and 38% sucrose. Fill carefully up with water to 13 mL.
2. Centrifuge at 100,000 × g for 3 h at 4 °C and subsequently collect the Golgi fraction (1) floating as boundary phase between 34% and 38% sucrose layers.
3. Adjust the collected Golgi fraction to 42% sucrose density again, and then apply 1-2 mL to the second discontinuous sucrose gradient consisting of 1 mL layers of 26%, 30%, 34% and 38% sucrose. Fill carefully up to 13 mL.
4. Centrifuge at 100,000 × g for 3 h at 4 °C and collect the Golgi fraction (2) floating as boundary phase between 34% and 38% sucrose layer.

By floating centrifugation twice using long narrow 13 mL tubes, highly purified Golgi can be isolated from the microsome. The Golgi fraction (2) is recovered and subjected to assays and blotting analyses. All sucrose concentrations based on w/w.

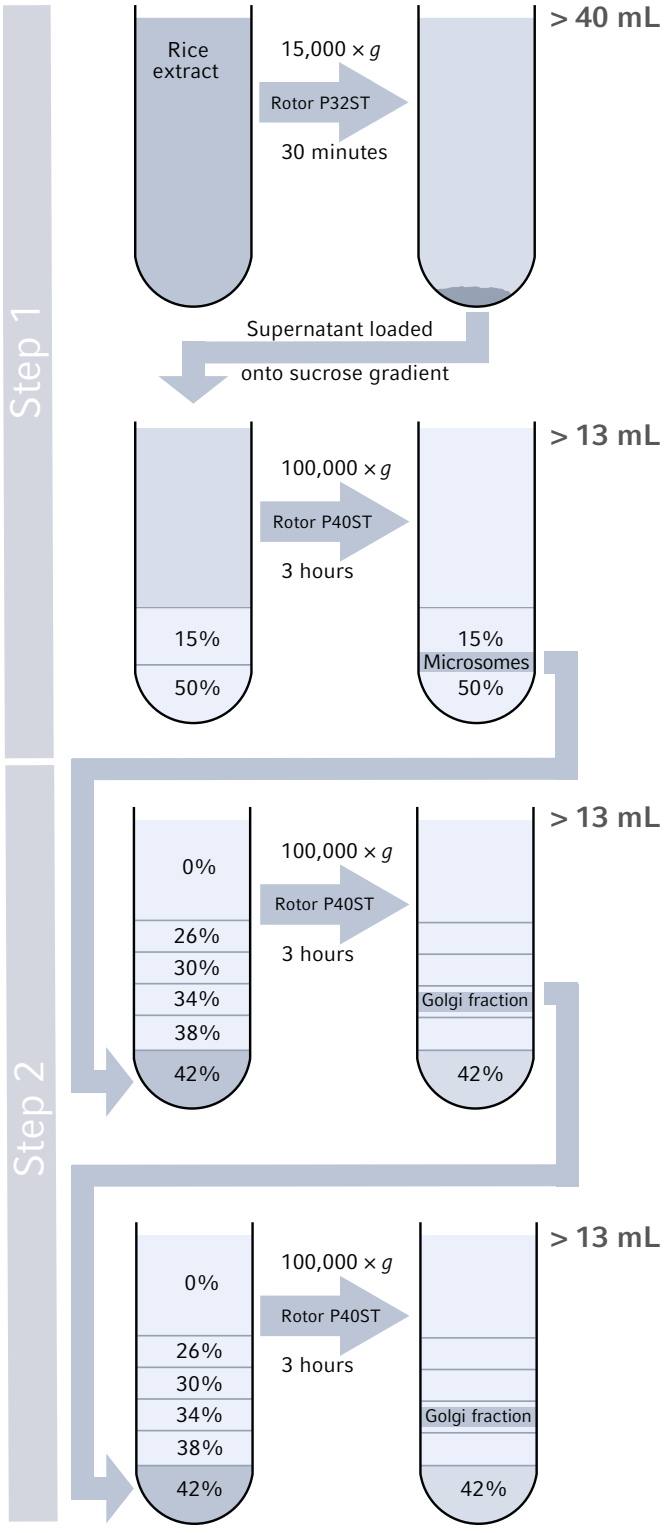


Figure 3: Isolation of Golgi bodies by a sequence of discontinuous sucrose gradient centrifugation steps. High purity is achieved by two separate floating steps.

## RESULTS AND DISCUSSION

The use of discontinuous density gradient centrifugation is a standard method for isolating or enriching subcellular components. In most cases, differences in the sedimentation coefficient or specific densities are used to obtain a separation.

The characteristics regarding these parameters in organelle isolation applications are mainly defined by the composition of the respective membranes.

A clear separation is often a challenge, especially for Golgi bodies, closely connected with other membrane systems [2]. Hence, highly purified Golgi membranes are essential for the analysis and investigation of, for example, the Golgi proteome [1] or specific proteins within the organelle.

Here, we describe an effective method using two different swing-bucket rotors in combination with successive discontinuous sucrose density gradient centrifugation steps to obtain high-quality isolates of Golgi bodies.

After removing the cell debris in the first centrifugation step, the supernatant is loaded onto the first discontinuous sucrose gradient (see Figure 3). Between the 15% and 50% sucrose phases, a fraction of microsomes is accumulated. This fraction is used for further purification by two steps of floating discontinuous sucrose gradients where the Golgi bodies accumulate between the 34% and 38% sucrose phase of the gradient (Figure 3). It was shown by Asakura et al. [4] that the purity of the Golgi body fraction was improved significantly after the second floating step. The quality of the Golgi fraction can be checked by the presence of marker enzymes like UGPase (Uridindiphosphate-Glucose-Pyrophosphorylase: Cytosol), RbcL (Ribulose bisphosphate carboxylase large chain: plastid), COXII (Cytochrome c oxidase subunit 2: mitochondria), and ARF (Adenosyl-Ribosylation-Factor: Golgi) by immunoblot analysis [1].

## CONCLUSION

The use of the combination of the two rotors P32ST and P40ST is ideal for the isolation of Golgi. It allows the shift from higher (40 mL) to lower volumes (13 mL) with high performance. The special long and narrow shape of the Himac 13 mL PET tubes allows a longer floating distance, which increases the purity of the Golgi fraction. Besides, the top-loading rotor inserts ease the delicate handling of sucrose gradients and minimizes the risk of unintended mixing.

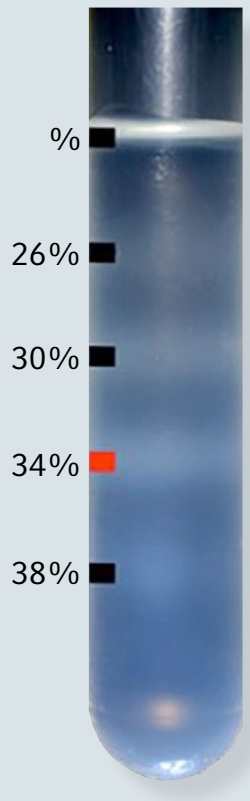


**Figure 4:** Sub fraction of the rice Golgi apparatus after multiple steps of discontinuous sucrose density gradient centrifugation. Golgi fraction between 34% and 38% sucrose solution indicated in red.

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