Lab Manager®

# CENTRIFUGE RESOURCE GUIDE

- Questions to Ask When Buying a Centrifuge
- The Basics of Centrifuge Operation and Maintenance
- In Centrifugation, Samples and Materials Must Work Together
- Safety in Centrifuges: Designed to Protect
- Streamlined Offerings Take Guesswork Out of Centrifuge Purchases

# Questions to Ask When Buying a Centrifuge

Centrifuges are used in laboratories to separate sample components based on size and density. The type of centrifuge required depends on your application, including the size and number of samples and the speed and g-force needed. Centrifuges can range in size from compact benchtop to large floor models and come with a variety of rotor options.

### What is my sample volume?

The volume of sample and, accordingly, the type of tube or plate being used will have a direct impact on which type of centrifuge is needed. Typically, benchtop models come in specialized formats that accommodate a narrower range of rotors and configurations. For samples and processes involving large or varying volumes, a floor standing model may be a better fit. The capacity and rotor configurations are usually more diverse in a floor standing model.

### Are my samples sensitive to changes in temperature?

As a centrifuge is engaged and begins to spin, heat will be generated by the friction of the rotor. If the samples are sensitive to changes in temperature, then a centrifuge that has refrigeration and temperature control options should be considered. Many vendors offer units with such capabilities, including CFC free options.

### Do I need to process blood and biological samples?

For clinical and blood banking procedures, there are a subset of specialized centrifuges available. Cell washers are ideal for blood banking purposes. These centrifuges are set to spin at a lower speed and are used to wash away cellular debris, extraneous proteins, and other debris from donor red blood cells. Cross-matching and other tests can then be performed on these blood samples. Clinical centrifuges are used to perform diagnostic examinations on blood, urine, and other samples. These specialized units spin at a lower speed and are typically low throughput.

### SAFETY TIP

Always make sure your samples are balanced before operating your centrifuge. Imbalanced samples can result in tube breakage, exposing you to aerosolized samples when you open the instrument's lid. More serious injuries can result from rotors that break due to sample imbalance. As an extra precaution, look for instruments that have auto-imbalance detection and will stop the centrifuge if an imbalance is detected.

# The Basics of Centrifuge Operation and Maintenance

What centrifuges are used for, how they work, how to choose a centrifuge, key safety tips, how to balance a centrifuge, and the basics of maintenance

### Part 1: What is a centrifuge used for?

Centrifuges are used in various laboratories to separate fluids, gases, or liquids based on density. In research and clinical laboratories, centrifuges are often used for cell, organelle, virus, protein, and nucleic acid purification.

An example of centrifuge use in a clinical setting is for the separation of whole blood components. Different assays necessitate serum or plasma, which may be obtained with centrifugation.

Serum is obtained by letting a whole blood sample clot at room temperature. The sample is then centrifuged, and the clot is removed, leaving a serum supernatant.

Unlike serum, plasma is obtained from whole blood that is not left to clot and contains serum along with clotting factors. To obtain plasma, a whole blood sample is collected in tubes treated with anticoagulants. Following centrifugation, cells are removed, and plasma supernatant remains.

### Part 2: How does centrifugation work?

#### Principles of centrifugation

A centrifuge is used to separate particles suspended in a liquid according to particle size and density, viscosity of the medium, and rotor speed.

Within a solution, gravitational force will cause particles of higher density than the solvent to sink, and those less dense than the solvent to float to the top. Centrifugation takes advantage of even minute differences in density to separate particles within a solution.

As the rotor spins around a central axis, it generates a centrifugal force acting to move particles away from the axis of rotation. If the centrifugal force exceeds the buoyant forces of liquid media and the frictional force created by the particle, the particles will sediment.

#### Centrifuge rotor types

There are two very common rotor designs: fixed angle and swinging bucket. The fixed angle rotor is designed to hold tubes in a fixed position at a fixed angle relative to the vertical axis of rotation (up to about 45°). Centrifugation will cause particles to sediment along the side and bottom of the tube. The swinging bucket design allows the tubes to swing out from a vertical resting position to become parallel to the horizontal during centrifugation. As a result, sediment will form along the bottom of the tube.

Fixed angle rotors are ideal for pelleting applications either to remove particles from a suspension and discard the debris or to recover the pellet, whereas swinging bucket rotors are best for separating large volume samples at low speeds and resolving samples in rate-zonal (density) gradients.

## Part 3: How do you choose a centrifuge?

### Centrifuge speed

Centrifuges may be classified based on maximum speeds, measured as revolutions per minute (RPM). Speeds range from 0-7,500 RPM for low-speed centrifuges, all the way to 20,000 RPM or higher.

Centrifuge rotor speed is often expressed as RCF in units of gravity (x g) for various procedures. However, many centrifuges display speed as revolutions per minute (RPM), necessitating conversion to ensure the correct experimental conditions. The following formula is used to convert RPM to RCF, where R is the rotor radius (cm) and S is the speed (RPM):

$g = (1.118 \times 10\text{-}5)\ R\ S^2$

### Centrifuge size

Centrifuges are available as various benchtop or floor-standing models.

Floor-standing models offer greater sample capacity and can achieve high speeds. Superspeed centrifuges can achieve a maximum g-force (relative centrifugal force, RCF) of over 70,000 x g, and ultracentrifuges often used for DNA or RNA fractionation, can achieve up to 1,000,000 x g. For large-capacity, low-speed applications, low-speed centrifuges reaching approximately 7000 x g are available.

Benchtop models have a smaller footprint, and general-purpose models are ideal for a wide range of applications. There are many benchtop models available, including high-speed, microcentrifuge, clinical, and cell washer models. Clinical benchtop models and cell washers typically operate at lower speeds, and are suited to diagnostic applications, and washing debris from red blood cells.

### Centrifuges for different applications

It is essential to select a centrifuge that is suited to the specific application. When purchasing a centrifuge, it is important to consider the following questions:

- What sample volumes are you working with? For processes involving large or varying volumes, a floor-standing model with higher capacity and different rotor configurations may be the best solution.
- Are samples temperature sensitive? If so, a centrifuge with refrigeration and temperature control options is required.
- Will the centrifuge be used for processing clinical or blood banking samples? Cell washers or clinical models are available for these specific applications.
- How much laboratory space is available vs the centrifuge footprint?
- What is the maximum g-force the centrifuge is capable of generating? Low-speed centrifuges are ideal for separating whole cells, while ultracentrifuges are necessary for separating DNA and RNA.

## Part 4: What safety precautions should be taken when working with a centrifuge?

### Ensure a sturdy, level worksurface

Always ensure the centrifuge is on an appropriate surface prior to operation.

### Balance the centrifuge

Running an unbalanced centrifuge may cause significant damage and injure the operator and other laboratory personnel. The total mass of each tube should be as close as possible- this becomes increasingly important at very high rotor speeds. Balancing masses to the nearest 0.1 gram is advisable, and it is important to balance tubes by mass, not volume. For example, do not balance a sample consisting of liquid with a higher or lower density than water with an equal volume of water.

### Do not open the lid while the rotor is moving

Many centrifuges have a "safety shutoff". However, this will only stop power to the rotor, which will still spin due to its own inertia for some time until it is slowed to a stop by friction.If the centrifuge is wobbling or shaking, pull the plug.

A little vibration is normal, but excessive amounts can mean danger. First, double check that the tubes are correctly balanced. If this does not resolve the issue, do not operate the centrifuge until it has been serviced by the manufacturer or dealer.

## Part 5: How do you balance a centrifuge?

### Why you need to balance a centrifuge

Prior to starting the centrifuge, it is necessary to load it correctly. Balancing the centrifuge prevents potential damage to the instrument and is crucial for safe operation.

### How to balance a centrifuge

1. Ensure all sample tubes are evenly filled. If additional tubes are required for balancing, fill them with water or a liquid of similar density to the sample, and ensure the mass is balanced to the nearest 0.1 grams.
2. For each tube inserted in the rotor, add a tube of equal

weight directly opposite it. This will ensure the center of gravity remains in the center of the rotor.

3. Rotate the rotor 90° and add two additional tubes directly opposite one another.

4. Repeat.

### How to balance 3 tubes, 5 tubes, or 7 tubes in a centrifuge with 12 positions

There are two ways to balance three tubes. The first option is to insert three sample tubes next to each other and create three balance tubes to be situated directly across from the sample tubes.

Alternatively, three sample tubes may be spaced evenly around the rotor.

To balance five tubes, create one balance tube and place two sets of three tubes across from each other.

To balance seven tubes, create one balance tube and place two sets of four tubes across from each other.

## Part 6: How do you maintain a centrifuge?

### Centrifuge care and maintenance

A few simple steps can keep a centrifuge functioning properly and reduce the risk of damage or injury.

- Keep the centrifuge properly lubricated. O-rings are the main source of protection against sample leakage and must be lubricated prior to installation of a new rotor or following cleaning. Any threaded components should also be cleaned regularly and lubricated with an approved grease to ensure proper operation and to prevent cross-threading and corrosion.

- Ensure all users are aware of how to properly operate the centrifuge, including ensuring buckets are properly seated in their pins, balancing tubes in the rotor, operating rotors within stated guidelines for speed and maximum compartment mass, and avoiding scratching the rotor.

- Inspect critical components, and look for signs of wear including scratches, or effects of chemical exposure on the rotor.

- Pay close attention to noise, vibration, shaking, or grinding and stop the unit immediately if this occurs.

### Centrifuge cleaning

Regularly clean the centrifuge with neutral cleaning solutions (alcohol or alcohol-based disinfectant) applied with a soft cloth to rotors and accessories. Daily cleaning should include the interior portion of the centrifuge, the rotor chamber, and surfaces with electronic components, such as touchscreens and keypads.

It is important to be aware of the different types of samples used with the centrifuge and any specific products or protocols necessary for cleaning spills.

# Product Spotlight

## A New Spin on Centrifugation

Over 50 years of innovation in centrifugation have led to the development of Eppendorf's new Centrifuge 5910 R, providing the ultimate versatility and capacity in a benchtop centrifuge. Its powerful, state-of-the-art refrigeration system keeps your samples safe from unwanted temperature fluctuations.

**Learn More**

# In Centrifugation, Samples and Materials Must Work Together

It's worth the trouble to match the optimal centrifuge tubes with a particular application

by Mike May, PhD

Although nearly every lab uses a centrifuge for some processes, the technique for every application varies—especially when it comes to the tubes. In short, the sample being spun down must be compatible with the material used to make the tube. In a perfect world, the key constituents of a sample do not interact with the tube, but that's not always how it works. It's worth the trouble to match the optimal centrifuge tubes with a particular application.

It's not just the sample of interest that matters when selecting an appropriate tube, but what's in the sample as well. That might include a solvent or something to form a gradient during centrifugation. With salt solutions, compatibility doesn't present much of an issue, if any. An alkaline solution, though, is not compatible with a polycarbonate tube. As a general rule, tubes should be tested for compatibility with a desired sample and protocol. Even the g-force or run time can impact how the tube and sample interact. For high-temperature centrifugation, for instance, stainless-steel or glass tubes work the best.

### Swaps for samples

The type of sample being used, and the centrifugation protocol can necessitate swapping one kind of centrifuge tube for another. For example, DNA sticks to some materials, and most of the nucleic acid processing works best in polyallomer tubes.

There are other ways centrifuge tubes can capture some of a sample. Scientists have investigated whether laboratory materials affect the analysis of perfluorooctanoic acid (PFOA), which is used in a range of industrial processes. This chemical is being replaced in some applications because of health concerns. The group of scientists tested the loss of PFOA solutions in various labware, including glass and plastic centrifuge tubes. The loss was significant in tubes made of polypropylene, polystyrene, polycarbonate, and glass—as much as 45 percent, 35 percent, 31 percent, and 24 percent, respectively. The study highlights the analytical bias that can occur because of sorption losses during routine procedures and demonstrates the importance of testing labware for specific PFOA analytes before experimental use.

Sometimes, scientists select centrifuge tubes made from a combination of materials. As an example, Dohan Ehrenfest of Chonnam National University (Gwangju, South Korea) and a team of scientists used glass-coated plastic tubes to centrifuge samples of leukocyte- and platelet-rich fibrin (L-PRF), which is a platelet concentrate used in surgical and dental treatments. Their findings indicate that "the centrifuge characteristics and centrifugation protocols impact significantly and dramatically the cells, growth factors, and fibrin architecture of L-PRF."

Ultimately, identifying the right centrifuge tubes for specific samples and processes takes some testing. The resistance guidelines make a great place to start. Plus, the manufacturer should include information about chemical resistance and durability for specific tubes. Even with this, some pretesting is the only way to be sure that specific centrifuge tubes serve the intended purpose.

# Safety in Centrifuges: Designed to Protect

In large part, centrifuge problems arise from user error rather than from instrument failure.

by Mike May, PhD

**From rotor sensing to vibration-reduction, new platforms protect you**

An online search for centrifuge accidents turns up some images that look like war scenes. In large part, centrifuge problems arise from user error rather than from instrument failure. For example, rotor incidents usually involve errors in connecting and tightening the centrifuge rotors onto the drive shafts, improperly balancing rotors, or using old and corroded rotors beyond their lifespans. Over-speed or over-use can also lead to tube failures. Many of today's devices help users avoid these problems.

To be safe, today's centrifuges provide several features, such as rotor recognition. This feature allows the centrifuge to detect which rotor is installed and check that the rotor's maximum speed is not exceeded by the program.

The centrifuge can also monitor other aspects of the rotor during operation. For example, integrated imbalance sensors and system shut down software allow centrifuges to quickly detect and reduce problems caused by improper rotor balancing by shutting down the system before the instrument reaches a critical imbalance condition.

**Key constraints**

When spinning something at high speeds, vibrations become the enemy. Old centrifuges are loud, vibrate when they start, and shake the entire bench, leading to safety issues.

New centrifuges fix that problem. For example, they offer shock-absorbing features that limit vibration.

Other important safety features can depend on the intended use. In some instances, for example, the samples being centrifuged really need to be kept away from the scientists. This arises in work like vaccine development, where dangerous organisms or components are used. Safety isn't just about the centrifuge, but also the labware being used. For example, canisters are available that are clear from top to bottom so you can see through them. When you take them out of the centrifuge bucket and move them to the fume hood, you can see if a tube has broken or if there's a leak before opening it. Other manufacturers offer one-handed sample protection that is simple to operate for all laboratory users and eliminates multi-turn screwcaps and high-pressure clips.

**Shopping for safety**

If a quarter-century-old centrifuge in your lab needs replacing, a couple of tips might simplify your shopping list. For one thing, make sure to buy one with the appropriate safety certifications, like UL/ CSA. Designations indicate that the centrifuge has been designed, tested, and manufactured to rigorous technical and safety standards. Any biocontainment lids should also be certified by an independent body.

So when you go centrifuge shopping, make sure that the vendor can prove that the safety features do the job.

# Streamlined Offerings Take Guesswork Out of Centrifuge Purchases

**Centrifuge providers have eliminated a lot of the guesswork and streamlined their offerings through a combination of adaptability, ease of use, and vastly improved technology.**

by Brandon Cook, PhD

Put yourself in the mindset of a new investigator beginning your own biomedical research lab. Most likely, your CV, expertise, and discoveries are inadequate predictors of your continued and future success. Suddenly, your career hinges on your ability to behave like an executive: making a first hire of a competent technician, planning an expansion during your start-up funding period, and implementing capital purchases that will maximize efficiency and stretch your budget.

First, you will require a suite of expensive equipment specific to your discipline. Regardless of your field of study, you will also need reagents, materials, and equipment ubiquitous throughout the life sciences. In particular, you will need a range of centrifuges with the capacity and versatility appropriate to your proposed benchmarks that will ensure the growth and success of the lab. That vision of success probably looks something like going from just you and a tech struggling at year zero to an efficient and productive workspace of seven or eight students and postdocs by the end of year three. Therefore, you will probably purchase one or two large tabletop centrifuges with swing buckets, one or two benchtop microcentrifuges, two temperature-controlled microcentrifuges, and small personal centrifuges for quick spins. There is a lot of potential for variation, depending, for instance, on whether your studies involve exosome profiling or virus collection, which would require capabilities limited to ultracentrifuges.

Your head is probably spinning by now, but you can rest easy. To a large extent, centrifuge providers have eliminated a lot of the guesswork and streamlined their offerings through a combination of adaptability, ease of use, and vastly improved technology.

For starters, providers are a lot more experienced with the lab start-up phase than you are. Although they do not commonly offer specific start-up packages, larger providers offer their main product ranges with all available rotors, attachments, and inserts under single part numbers to avoid costly and confusing a la carte shopping. With the exception of floor-model ultracentrifuges, units are typically designed to be "plug and play," with little or no specialized knowledge required for immediate use. Increased capacity and decreased negative space have reduced energy and size footprints, improving efficiency and savings over the long term.

Additionally, material improvements to interchangeable parts have extended life spans and reduced the need for repair and maintenance contracts. For instance, the move from aluminum to carbon fiber rotors has diminished corrosion problems and allowed for longer warranties, and fast, foolproof rotor-swapping mechanisms have improved safety while helping you avoid the shame of having to find stronger hands than yours to help unscrew the wrong rotor.

These improvements come with an associated initial cost, but if you plan appropriately, the savings over the long term can be substantial. For example, the growth in functional assays using RNA and protein samples has prompted a marked shift

to refrigerated microcentrifuges. There is a greater initial cost than buying a regular micro and sticking it in the cold room; however, the efficiency and consistency of sample collection will be improved with dedicated temperature control, compared with a motor that generates unregulated heat while only the outer housing sits at 4 degrees. Also, you can offset the cost by forgoing refrigeration for large tabletop machines—cell cultures will remain viable when spun at low speed and room temperature.

In an era of tightened National Institutes of Health budgets, the market for used equipment, available from some providers, has also grown substantially. Regardless, the diverse menu of ready-to-use options takes a potentially bewildering large purchase and makes it relatively painless and straightforward.

# Featured Manufacturers

eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling. Its product range includes pipettes and automated pipetting systems, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO2 incubators, shakers, and cell manipulation systems. Consumables such as pipette tips, tubes, plates, and disposable bioreactor vessels complement the range of highest-quality premium products.

www.eppendorf.com