

Centrifugation

PRINCIPLES OF CONTINUOUS FLOW CENTRIFUGATION

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To process material in conventional centrifugation rotors, the following steps must be performed:

1. Load the rotor with sample,
2. Accelerate to operating speed,
3. Run for a specific period of time,
4. Decelerate to a stop,
5. Unload the sample.

These steps must be repeated until all sample is processed. If the quantity of material to be processed is large, its sedimentation rate low, the acceleration/ deceleration time of the rotor long, and the rotor capacity small, conventional processing of large volumes of material will be labor intensive and very time consuming.

Continuous flow centrifugation is a laboratory time-saver whereby large volumes of material can be centrifuged at high centrifugal forces without the tedium of filling and decanting a lot of centrifuge tubes, or frequently starting and stopping the rotor. For example, 10 L of liquid containing 500 S particles can be processed by continuous flow methods in 4 h or less, depending on the rotor selected. [Note: The sedimentation coefficient is usually expressed in Svedberg units, where $1 \text{ S} = 1 \times 10^{-13}$

seconds. Thus, the sedimentation coefficient of a particle measured at 500×10^{-13} seconds is said to have a value of 500 S. The velocity of a particle in a centrifugal field can be defined as $s = v/\omega r^2$, where s is the sedimentation coefficient, v is the velocity of the particle in centimeters per second, ω is the angular velocity of the rotor in radians per second, and r is the distance from the axis of rotation in centimeters. Sedimentation coefficients, as used here, are s_{obs} (values observed for the particular solvent system and temperature used) rather than $s_{20,w}$ values for water at 20°C.] This same operation would require 12–21 h by conventional batch-type centrifugation. This combination of high centrifugal force and high throughput makes continuous flow processing particularly useful for the large-scale collection of viruses (either for research purposes or for the preparation of commercial vaccines), the sedimentation of bacteria, and the pelleting of subcellular fractions. However, continuous flow centrifugation need not be confined to biomedical applications. It should be considered whenever particles of any kind with sedimentation coefficients of 50 S or larger must be routinely separated from fluid volumes of 2 L or more.

Continuous flow rotors substantially minimize material processing time for two reasons:

1. They have short pathlengths to reduce overall pelleting time. Hence, they efficiently pellet solids out of a sample stream and facilitate a rapid flow of material through the rotor.
2. They have large capacities. Therefore, they do not need to be started and stopped as often as conventional rotors. This saves time by reducing sample handling and reducing the time lost in waiting for rotor acceleration/deceleration between runs.

Qualifying the Sample

Continuous flow rotors are of greatest benefit over conventional rotors when the sample has the following properties:

1. The sedimentation coefficient of the particles to be collected is greater than 50 S. Because the rotor has high pelleting efficiency, solid material can be separated from the liquid medium faster than with a swinging bucket or fixed angle rotor.
2. The sample solid/liquid ratio is low (5-15%). Above a solid/liquid ratio of 15%, the rotor tends to be overefficient; *i.e.*, it pellets so quickly that it fills immediately. This means that it must be stopped for unloading the pellets so often that too much time is lost in accelerating/decelerating the rotor and cleaning it between runs. Conversely, if the sample contains little solid material, the rotor will operate for long periods of time, processing large volumes of material between shutdowns.

Continuous Flow Operation

Both Beckman Coulter continuous flow rotors, the CF-32 Ti and the JCF-Z, operate on a continuous flow, batch-processing basis. That is to say, a batch of particle-containing liquid flows continuously into the rotor, which is running at the selected operating speed. Particles sediment out of the flowing stream that then emerges as a particle-depleted effluent. This process continues until the particle-containing capacity of the rotor is reached, or until the starting material (sample) is completely processed. Thus, the amount of starting material that can be handled in one run is governed by the concentration of the particles it contains as well as its volume. The sediment, the particle-depleted effluent, or both can be collected, just as one collects the sediment and/or supernatant after differential centrifugation in conventional fixed angle or swinging bucket rotors.

Both the CF-32 Ti and JCF-Z rotors consist of a bowl, a solid core that is placed inside the bowl, and a lid (see Figure 1). Radial channels for the flow of liquids pass through the core. There are four milled slots on the top surface of the core through which liquids flow for loading and sometimes unloading the rotor. The continuous flow mode of operation is made possible by the rotating seal assemblies that permit the fluid-bearing lines to remain attached to the rotors during operation. These seals are designed to prevent exposure of the sample to environmental air, a feature that minimizes possible contamination of the sample. For loading and unloading, the flow of fluid through the rotor can be reversed by simply switching the fluid lines.

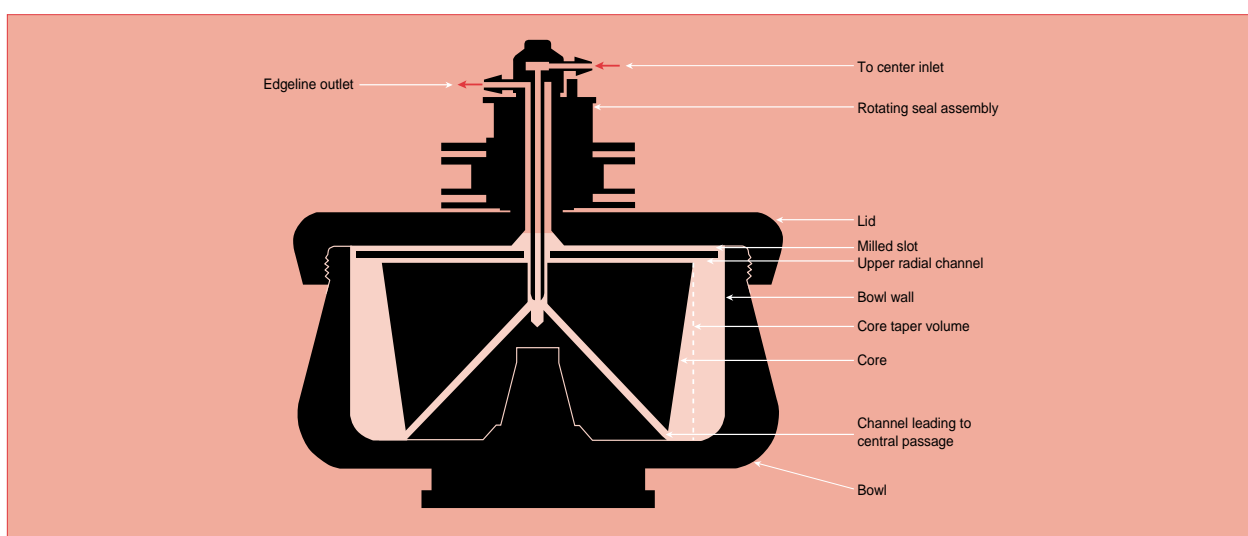


Figure 1. Cross section of a continuous flow rotor

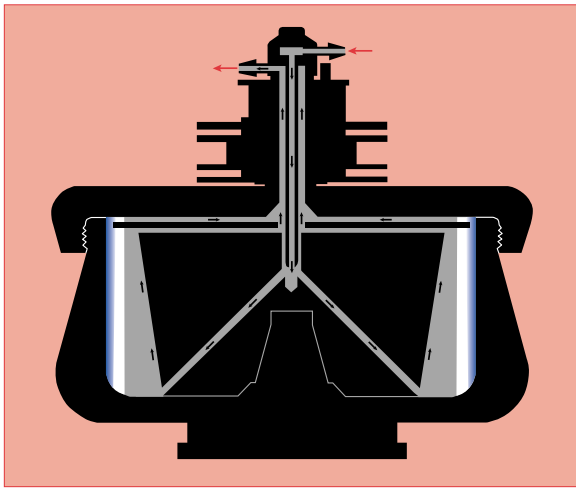


Figure 2. Continuous flow centrifugation. The arrows on the diagram indicate the direction of liquid flow during continuous flow operation. The sample is pumped in through the center inlet to the bottom of the core. The flow rate is adjusted so that the particles of interest have time to become trapped in the gradient or cushion (or pelleted on the rotor wall) during the time required to move from the bottom of the core to the upper radial channel.

The rotor is prepared for centrifugation while spinning at low speed. Buffer solution, cushion, or discontinuous layers of a gradient material such as sucrose are pumped in through the edge line. Then the rotor is accelerated to operating speed, the fluid lines are switched, and the particle-containing liquid is routed through the center inlet of the seal with the same pump. It is delivered through the channels in the rotor core to the bottom of the rotor bowl. This direct, sealed pathway virtually eliminates undesirable foaming (see Figure 2). As the flowing liquid moves upward along the core taper toward the top of the bowl, sedimentation of particles toward the bowl wall takes place. The particle-depleted effluent travels through the upper radial channel and leaves the rotor via the outlet (edge) line in the seal assembly.

Separation Techniques

Particles may be concentrated in one of three ways: by pelleting onto the wall of the rotor bowl, by sedimentation onto a cushion of dense liquid such as sucrose, or by banding in a gradient. The first two methods are most useful for either harvesting the particles, or for recovering the particle-depleted effluent. Whenever it is necessary to separate a particle from contaminants of different densities, banding will give the best results.

Pelleting and Clarifying

Pelleting is suitable for collecting particles that won't be damaged by being pressed against the wall of the rotor. It is the fastest continuous flow method and can handle large volumes of starting material.

While operating at low speed, the rotor is filled with buffer solution or starting material. Then the rotor is accelerated to the selected run speed, and starting material is pumped through the center line of the seal. The run is continued until all the material has been processed, or the maximum pellet capacity of the rotor has been reached. (The latter case may be apparent when the effluent emerging from the rotor becomes turbid.) At this point, rotor speed is maintained long enough for all sedimenting particles to reach the rotor wall, while buffer solution or water is pumped through the center line. Then the rotor is decelerated to rest, the supernatant remaining in the rotor is decanted, and the pelleted material is scraped off the rotor wall. Sometimes, two stages of flow-through centrifugation can be employed—a first-stage clarification at low rotor speed to remove unwanted large particles, followed by a second pass of the effluent through the rotor running at high speed to collect small particles. A virus-containing culture fluid, for example, may be cleared of cellular debris in this manner. The clarified effluent can then be reprocessed by pelleting or banding to concentrate the virus particles.

Sedimenting onto a Cushion

Particles that might lose biological activity if pelleted (some viruses, for example) can be sedimented onto a cushion of a dense solution such as sucrose. A cushion should be used whenever it seems more convenient to collect the particles in solution rather than having to scrape a pellet off the rotor wall. The particle-bearing capacity of the rotor will, of course, be reduced by the presence of a cushion.

For this type of run, the rotor is loaded while operating at low speed (see Figure 3). Buffer or water is introduced first through the edge line and over the milled slots on top of the core. Then the cushion (a 60% by weight sucrose solution, for example) is loaded in the same manner. It enters at the periphery of the rotor where it is held against the wall by the centrifugal force field because it is more dense than the water or buffer. At this point, flow lines are switched, and buffer is pumped through the center line as the rotor is accelerated to operating speed. Once operating speed is reached, starting material is pumped through the center line. When sedimenta-

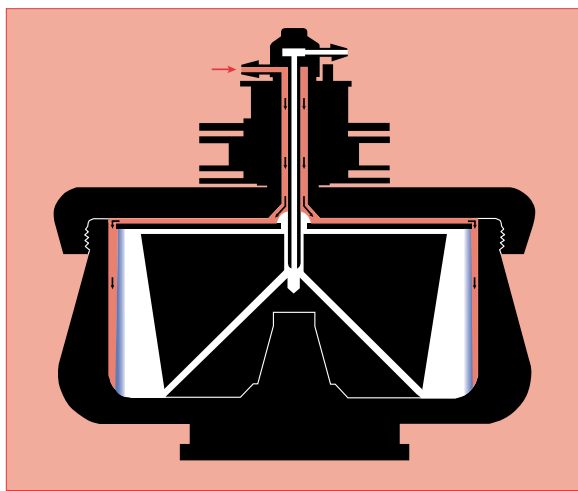


Figure 3. Loading a cushion or gradient. The arrows indicate the direction of liquid flow during loading. With the rotor turning at low speed, the cushion or gradient (light end first) is pumped in through the edge line. Air is displaced through the center inlet. The cushion or gradient is held against the rotor wall by centrifugal force.

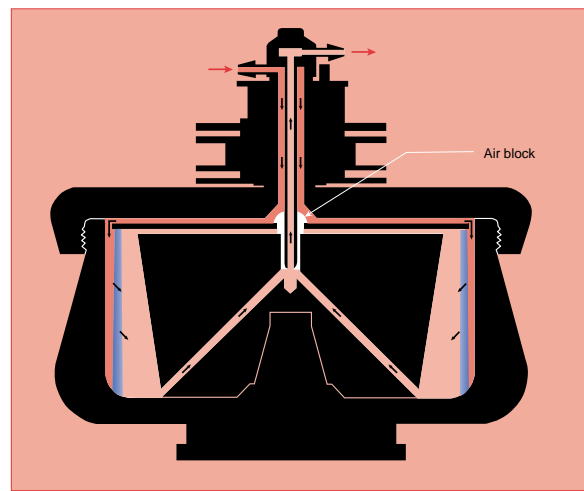


Figure 4. Unloading a cushion or gradient. When the particles of interest are contained in a cushion or gradient, it is necessary to unload without mixing the rotor contents. With the rotor turning at low speed, air is introduced through the edge line to form a bubble blocking the upper radial channels. A dense solution is then pumped in through the edge line, forcing the cushion or gradient out through the center.

tion is completed, unloading, like loading, is done with the rotor turning at low speed. Air is introduced through the edge line to block the upper radial channels as shown in Figure 4. (This prevents displacement fluid from entering these channels and disturbing the particle-laden cushion during the unloading process.) Then the rotor is unloaded by pumping a dense solution through the edge line and over the milled slots, thereby displacing the rotor contents through the center line.

Banding in a Gradient

This method is most commonly used for the purification of viruses. Because of the short sedimentation pathlength in the continuous flow rotors (about 10 mm), it is not necessary to use a preformed linear gradient. Step concentrations will diffuse enough to become linear during the loading and acceleration process. Usually, two or three concentration steps of a gradient material, such as sucrose solution, are selected that encompass the density of the particles to be banded. The last and most dense solution acts as a cushion to prevent sedimentation to the rotor wall. The short pathlength makes it possible to band particles quite rapidly. However, depending on the buoyant densities of the particles, it may be difficult to completely resolve and collect multiple bands from within such a short gradient.

Operation is quite similar to that described under *Sedimenting onto a Cushion*. While the rotor is operating at low speed, fluids are loaded through the edge line in this order: buffer solution or water, followed by the step gradient (light end first), and the cushion last. The remaining sequence of operation is the same as described in the preceding section. In this case, however, sedimentation may be allowed to continue as long as necessary for banding of the particles.

Selecting a Flow Rate

The sedimentation coefficient of the particles to be collected governs the selection of flow rate and operating speed. Small particles require either a higher centrifugal force field or more time to sediment than larger ones. The aim is to select an operating speed that will generate a centrifugal force high enough to sediment the particles of interest, and a flow rate low enough to provide time for these particles to sediment out of the flowing stream before it leaves the rotor. For most efficient operation, one tries to use a flow rate as close as possible to the theoretical maximum. (Nomograms that portray the relationship between theoretical maximum flow rate and rotor speed for particles of known sedimentation coefficients can be found on pages 10 and 11.)

Method A

The nomograms have been generated from the following equations, which may be used for determining an approximate flow rate, F , or rotor speed for specific samples.

$$F = \pi s_r h \omega^2 \left(\frac{r_{\max}^2 - r_i^2}{\ln(r_{\max} / r_i^h)} \right) \quad (1)$$

where $\pi = 3.1412$

$h =$ height of the rotor core

$\omega = 0.10472 \times \text{RPM}$

$r_{\max} =$ maximum radius of the core

$$r_i = \frac{r_t - r_b}{\ln(r_t / r_b)}$$

$r_t =$ radius at top of core

$r_b =$ radius at bottom of core

$s_r =$ sedimentation coefficient of particle, in Svedberg units, adjusted for run conditions.

F is expressed in mL/min. For the JCF-Z Standard Pellet Core, $r_t = 7.6$ cm, and $r_b = 7.1$ cm, reducing r_i to 7.35 cm and Equation (1) to Equation (2a):

$$F = 2.23 \times 10^{-10} s_r (\text{RPM})^2 \quad (2a)$$

For the Large Pellet Core, $r_t = 5.6$ cm and $r_b = 5.1$ cm. Thus, r_i becomes 5.35 cm and Equation (1) reduces to Equation (2b):

$$F = 1.69 \times 10^{-10} s_r (\text{RPM})^2 \quad (2b)$$

Note: The above equations assume that the density and viscosity of the liquid in which the particles are suspended are similar to water at 20°C. If this is not so, an adjusted sedimentation coefficient, s_r , should be calculated.

Before the nomogram or Equation (2) can be used, however, s_r must be calculated from the sedimentation coefficient or the diameter, D , of the particle of interest. If the diameter is known, use Equation (3):

$$s_r = \frac{D^2 (\rho_p - \rho_r)}{18 \eta_r} \quad (3)$$

where $\rho_p =$ density of the particles in g/mL

$\rho_r =$ density of the liquid containing the particles in g/mL

$\eta_r =$ viscosity of the liquid in mPa•s or in cp

If the sedimentation coefficient (*i.e.*, $s_{20,w}$) of the particle is known, it may be used to calculate s_r as follows:

$$s_r = s_{20,w} \left(\frac{\rho_p - \rho_r}{\rho_p - \rho_{20,w}} \right) \left(\frac{\eta_{20,w}}{\eta_r} \right) \quad (4)$$

Method B

A simple way to determine the flow rate for a continuous flow rotor is to use known k -factors to compute pelleting times from another rotor. The k factor is a constant that is different for each rotor and is a measure of pelleting efficiency. It is derived from the equation:

$$k = \frac{\ln(r_{\max} / r_{\min})}{\omega^2} \times \frac{10^{13}}{3600}$$

where $r_{\max} =$ maximum radius from centrifugal axis

$r_{\min} =$ minimum radius from centrifugal axis

$\omega =$ angular velocity in radians/second
 $= 0.10472 \times \text{RPM}$

For example, if separation is performed in a JA-10 rotor, and the time to pellet in a full bottle run at full speed is 5 min, then this information can be used to determine the pelleting time in the JCF-Z rotor in the following way:

$$\frac{t_1}{t_2} = \frac{k_1}{k_2}$$

where $t_1 =$ time to pellet in the first rotor

$t_2 =$ time to pellet in the second rotor

$k_1 = k$ factor of the first rotor

$k_2 = k$ factor of the second rotor

Substituting the experimental values results in the following equation:

$$\frac{300}{t_2} = \frac{3700}{294}$$

or $t_2 = 24$ seconds to pellet in the JCF-Z rotor. If the volume of the JCF-Z rotor is 1000 mL, then we know we can pellet 1000 mL in 24 seconds, or we can use a flow rate of 41 mL/s (2.4 L/min). If the solid/liquid ratio of the sample is 5%, we can process 200 L of material (total time = 1:24 h) before shutting down the rotor for cleaning. Comparing the 84-min processing time to a JA-10 rotor, the JA-10 can process 1.5 L in 5 min; the time to process 200 L is 11:10 h. The continuous flow rotor is at least 8 times faster than a conventional large-volume rotor such as the JA-10.

Rotors, Cores, and Their Uses

There are two continuous flow rotors currently available from Beckman Coulter: the JCF-Z rotor and the CF-32 Ti rotor. The JCF-Z rotor has a maximum speed of 20,000 rpm and is designed for use in the J-21 series and J2-21 centrifuges. It has three interchangeable cores for continuous flow operation and two for zonal runs. These are: the Standard Core, the Large Pellet Core, the Small Pellet Core, the Reorienting Gradient Core, and the Zonal Core,



Figure 5. JCF-Z continuous flow rotor

respectively. Only the continuous flow cores will be discussed here. (See Figure 5 for a photograph of the JCF-Z rotor; its continuous flow cores are shown in Figure 6.) The CF-32 Ti rotor has one core (Figure 7) and is designed for continuous flow operation only. It has a maximum speed of 32,000 rpm and can be used in most Model L series ultracentrifuges (Optima™ XL series, L8, L5, L3, L2 series, and those Model L's classified as B).

The bowls and lids of both the JCF-Z and CF-32 Ti rotors are made of titanium. The cores are Noryl which can be used safely with most of the common density gradient media and buffers. (Consult the rotor or Model J2-21 centrifuge instruction manuals for a list of materials to which Noryl is resistant.) All but the JCF-Z Small Pellet Core are tapered and have four removable Noryl baffles attached that stabilize the liquid within the rotors during operation. These baffles divide the rotor interior. (Unlike the other cores, the Small Pellet Core has six cavities within the core itself in which pelleting takes place.)



Figure 6. JCF-Z continuous flow rotor and cores

The amount of space between the core and the bowl wall determines the total capacity of the rotor. Of this total capacity, a certain amount can be occupied by pellet layer, gradient, or cushion at the periphery of the rotor. The remaining capacity, next to the core, is called the taper volume. This is the region through which the starting material flows, and where sedimentation into a gradient or onto the cushion or rotor wall takes place.

The specifications of the various rotor-core configurations are summarized in Table 1.

JCF-Z Rotor

The JCF-Z rotor equipped with one of its continuous flow cores is suitable for the sedimentation of bacteria, the larger subcellular particles such as membranes, mitochondria, etc., and those viruses or other particles that have sedimentation coefficients of 500 S and higher. It is also used for clearing virus-containing culture media of cellular debris prior to final collection of the virus by pelleting or banding in the same rotor or the CF-32 Ti. When running at its maximum speed of 20,000 rpm, the JCF-Z with the Standard Core generates $32,000 \times g$ at the core bottom and $39,900 \times g$ at the inner surface of the bowl wall. It can be operated at flow rates between 3 and 45 L/h (the rotating seal assembly requires a minimum flow rate of 3 L/h). A High-Flow Seal Assembly is available for handling very large volumes where higher flow rates would be expedient. This accessory makes it possible to process up to 100 L/h.

This rotor can be used in all J2-21 and J-21 series centrifuges without modification. These centrifuges are refrigerated, but precooling the rotor is recommended whenever temperature control of the sample is critical. In this case, the fluids to be pumped through the rotor should be precooled also because they will be warmed to some extent as they



Figure 7. CF-32 Ti continuous flow rotor and core

travel through the pump tubing and the seal assembly. Sample reservoirs should be kept in ice or in a refrigerated water bath, and tubing lines should be kept as short as possible. The amount of the anticipated pellet, or the necessity to sediment onto a cushion or into a step gradient, determines which of the three JCF-Z continuous flow cores should be used.

The Standard Core has a total capacity of 660 mL. Of this volume, about 400 mL can be occupied by pellet, step gradient, or cushion. This core is suitable for pelleting against a cushion or the rotor wall, clarifying liquids, or banding of particles.

The Large Pellet Core is suitable for pelleting only. It has a total capacity of 1250 mL, of which approximately 800 mL can be occupied by pellet. This core is well-suited for centrifuging fluids that contain a large amount of solids; a solid-to-liquid ratio as high as 1:2 can be processed. Of course, a high solid-to-liquid ratio means that less material can be processed before stopping to unload the rotor.

The Small Pellet Core has a total capacity of 240 mL, of which about 200 mL can be occupied by pellet. It is designed for processing large amounts of material with a low solid-to-liquid ratio (water that contains algae or clay particles, for example). In order to minimize resuspension of sedimented material, this core has six individual cavities, each holding removable canoe-shaped containers in which the pellets are collected. This core, too, is designed for pelleting only.

The JCF-Z rotor has two other interchangeable cores for zonal separations: the Reorienting Gradient Core and the Zonal Core.

CF-32 Ti Rotor

The CF-32 Ti rotor can be used to concentrate particles with a wide range of sizes. Because of its high speed (32,000 rpm) it is particularly useful for banding viruses or other small particles that have sedimentation coefficients as small as 50 S. At maximum speed, it generates $86,100 \times g$ and $102,000 \times g$

Table 1. Beckman Coulter Continuous Flow Rotors and Cores

<i>Specifications</i>	<i>CF-32-Ti</i>	<i>JCF-Z</i>		
		<i>Standard Core</i>	<i>Small Pellet Core</i>	<i>Large Pellet Core</i>
Maximum rotor speed	32,000 rpm ¹	20,000 rpm	20,000 rpm	20,000 rpm
Maximum force				
at bottom of core	86,100 g	32,000 g	25,000 g	23,000 g
at top of core	91,950 g	34,000 g	25,000 g	25,000 g
at rotor wall	102,000 g	39,900 g	36,300 g	39,900 g
Total rotor capacity	430 mL	660 mL	240 mL	1250 mL
Maximum pellet capacity	330 mL	400 mL	204 mL	800 mL
Maximum flow rate				
Standard Seal Assembly	9 L/h	45 L/h	45 L/h	45 L/h
High Flow Seal Assembly	—	100 L/h	100 L/h	100 L/h
Maximum permissible density of contents at maximum speed ²	1.20 g/mL	1.45 g/mL	1.45 g/mL	1.45 g/mL
Permissible pH range for liquids	pH 4–10	pH 4–10	pH 4–10	pH 4–10
¹ After 1000 runs or 2500 hours of centrifugation, the CF-32 Ti must be derated to 29,000 rpm. ² If the density of the <i>heaviest</i> gradient fraction exceeds 1.20 g/mL for the CF-32 Ti, or 1.45 g/mL for the JCF-Z, the maximum permissible rotor speed must be reduced. Speed reductions can be calculated as follows: For the CF-32 Ti, maximum possible speed is $\sqrt{\frac{1.20 \text{ g/mL}}{\text{maximum density at rotor wall}}} \times \text{maximum or derated speed.}$		For the JCF-Z, the maximum available rotor speed is $\sqrt{\frac{1.45 \text{ g/mL}}{\text{maximum density at rotor wall}}} \times 20,000 \text{ rpm.}$ Speed reductions may also be required when sedimenting unusually heavy pellets, <i>i.e.</i> , liquids containing metal or clay particles, for example.		

at the core bottom and inner surface of the bowl wall, respectively. It operates at flow rates up to 9 L/h.

The total capacity of this rotor is 430 mL, of which about 330 mL can be occupied by pellet. This capacity is reduced when a cushion or step gradient is used, either of which will occupy about 300 mL. The ultracentrifuges that accommodate the CF-32 Ti rotor provide refrigerated operation. Because the seal assembly of the CF-32 Ti is equipped with a stainless steel water jacket, normal temperatures at the seal can be maintained at much lower flow rates than in the JCF-Z rotor. A tap water supply can be used for the water jacket unless a temperature of 10°C or lower at the seal assembly is necessary. In that case, a water cooler and pump should be used, and the starting material and rotor precooled as well.

Other Equipment Required

In addition to either the JCF-Z or CF-32 Ti rotor and its respective centrifuge, a peristaltic pump, capable of operating against a back pressure of 138 kPa (20 psi), is required for introducing cushions or step gradients into the rotor, as well as for pumping starting material. A pressure gauge to check flow rates between the pump and the seal assembly may also be helpful. A flow-through photometer is useful for monitoring the effluent to observe sample cleanout during pelleting, and for identifying banded material during unloading of cushions or gradients. Tubing lines in and out of the flow cell should have the same diameter as the rotor tubing. A fraction collector may also be desirable. Of course, it will be necessary to have sample and effluent reservoirs, as well as any refrigeration accessories required. Typical equipment setups are shown in Figures 8 and 9.

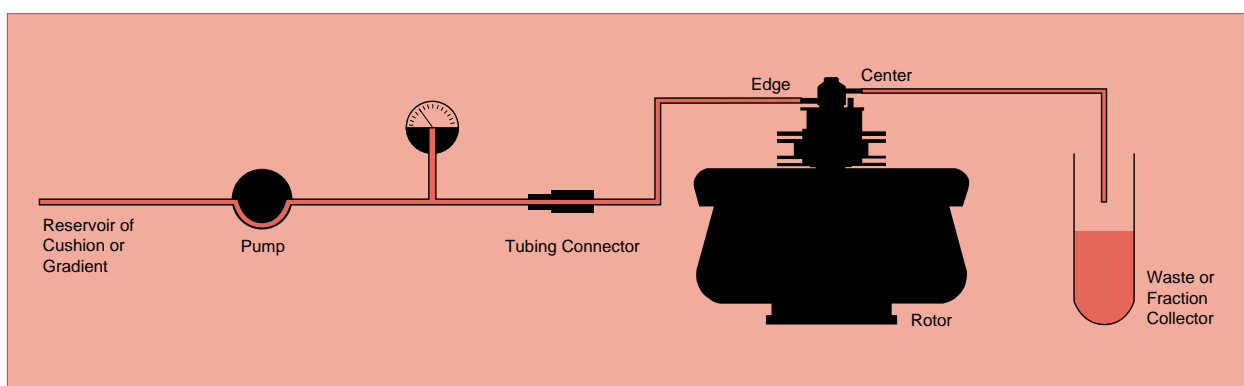


Figure 8. Equipment arrangement for loading and unloading cushion or step gradient

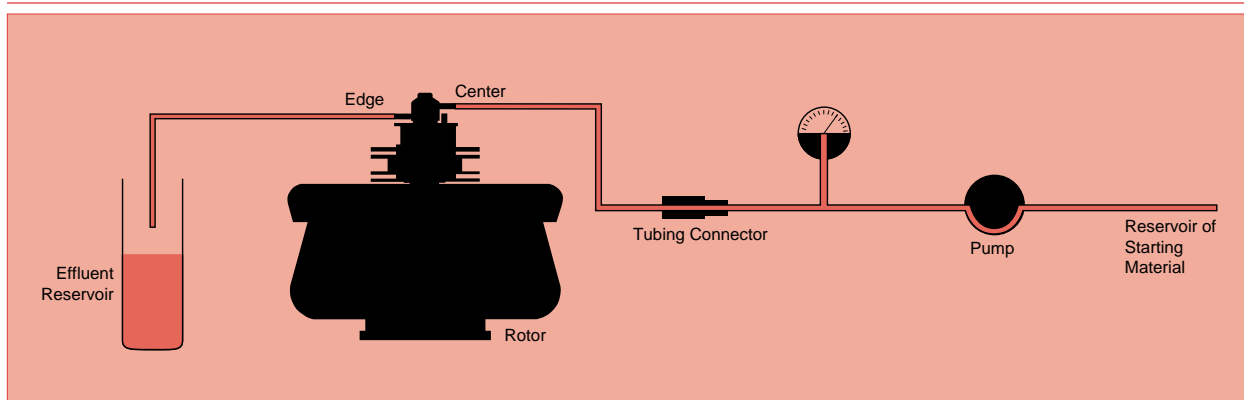


Figure 9. Equipment arrangement during continuous flow centrifugation

Typical Questions and Answers Regarding Continuous Flow Centrifugation

Q I frequently need to separate particles from large volumes of starting material, but I'm not sure if I need a continuous flow rotor or a zonal rotor. Which would be better for my application?

A If you have been successfully using conventional fixed angle or swinging bucket rotors to pellet your particles, with or without a cushion, a continuous flow rotor will enable you to scale up this process. This will also be true if you have been banding the particles isopycnicly, provided there are no contaminants which band near the same density. If you do need to resolve multicomponent samples, one of the zonal rotors would be a better choice. Although the sample handling capabilities of zonal rotors (20–200 mL) are considerably less than continuous flow rotors, they are 50–100 times greater than those of swinging bucket rotors.

Also, if you have been isolating your particles by rate-zonal sedimentation, or need to obtain sedimentation coefficients for your particles, a zonal rotor is what you need. Continuous flow rotors are not suitable for rate-zonal centrifugation.

Q I am currently using a fixed angle rotor for pelleting and would like to make the same separation by continuous flow methods. How do I determine the proper flow rate?

A In order to determine the necessary flow rate, you must first calculate an approximate sedimentation coefficient for the particle of interest. For this purpose, a rough estimate is sufficient and can be calculated as follows:

$$s = k/t$$

where s is the sedimentation coefficient in Svedberg units, k is the k factor (clearing factor) for the fixed angle rotor you are currently using (it can be found in your rotor manual), and t is the time (in hours) it takes to pellet the particles in that rotor. If your rotor was operated at less than maximum speed, determine s as follows:

$$s = (k/t) (\text{maximum rotor speed/reduced rotor speed})^2$$

The estimated sedimentation coefficient can now be used to obtain an approximate flow rate from the nomograms shown on pages 10 and 11.

Q How serious are air bubbles?

A Air bubbles will cause high back pressure. This is not a problem at low speed, as during loading, but at operating speed, the buoyancy of the bubble is multiplied by the relative centrifugal force. Such a bubble cannot be dislodged by inflowing sample. If the pressure is above 173 kPa (25 psig), follow the instructions given in the rotor manual for removing air trapped in the system. It may be necessary to install a bubble trap between the pump and the rotor connection to collect any air bubbles which may have formed in the sample due to outgassing.

Q How do I determine run conditions?

A Run conditions are a combination of speed and flow rate. Unless a sample particle is so small that it requires the highest possible speed with the lowest practical flow rate, there can be multiple speed/flow rate combinations that will retain particles of a given size class. Usually flow rate is chosen based on the amount of sample to be processed and how long you want that processing to take; speed then follows from that choice.

Q How long does it take to set up for a continuous flow separation?

A An experienced user will need about 30 minutes to prepare the equipment and about 10 minutes to dismantle it.

Q How do I get my sample out?

A Some samples, bacteria or yeast, for example, will pellet on the rotor wall. Remove the supernatant remaining with a large syringe or siphon and scrape out the pellet cake. If you are using the JCF-Z Small Pellet Core, simply remove the canoe-shaped containers and scrape out the pellet. Other samples, such as cell membranes or whole cells, will not adhere to the rotor wall but will form a very concentrated slurry with the liquid remaining in the bowl. In this case, an additional spin in a conventional rotor will yield a pellet.

Q How should I clean my rotor after use?

A After each use, all parts of the rotor and seal assembly that have come in contact with the sample should be rinsed in mild detergent, followed by distilled water. They should be dried with warm air. They may then be autoclaved at temperatures up to 125°C, except for the removable Noryl baffles on the core and the canoe-shaped containers of the Small Pellet Core. These may warp at this temperature. Moisture can reduce the lifetime of the JCF-Z bearings, so they should be placed in a vapor-tight container if autoclaved.

Q What about continuous flow centrifugation of pathogens?

A All Beckman Coulter continuous flow rotor manuals carry this warning: "The processing of large volumes of pathogenic materials in these rotors can produce extremely potent concentrations. Agents that are relatively harmless in their naturally occurring dilute state may become infectious when concentrated. Biological hazards must be carefully assessed and precautions taken." Beckman Coulter offers a modified centrifuge that can be used in conjunction with a Class I cabinet or a hood to minimize the exposure to aerosols. Remote control panels are available for most of the centrifuges, and Absolute High Efficiency Particulate Aerosol (HEPA) filters are also available for placing between the vacuum chamber and the vacuum pump. Consideration must be given to the air flow in the room, and your Laboratory Safety Officer should be consulted.

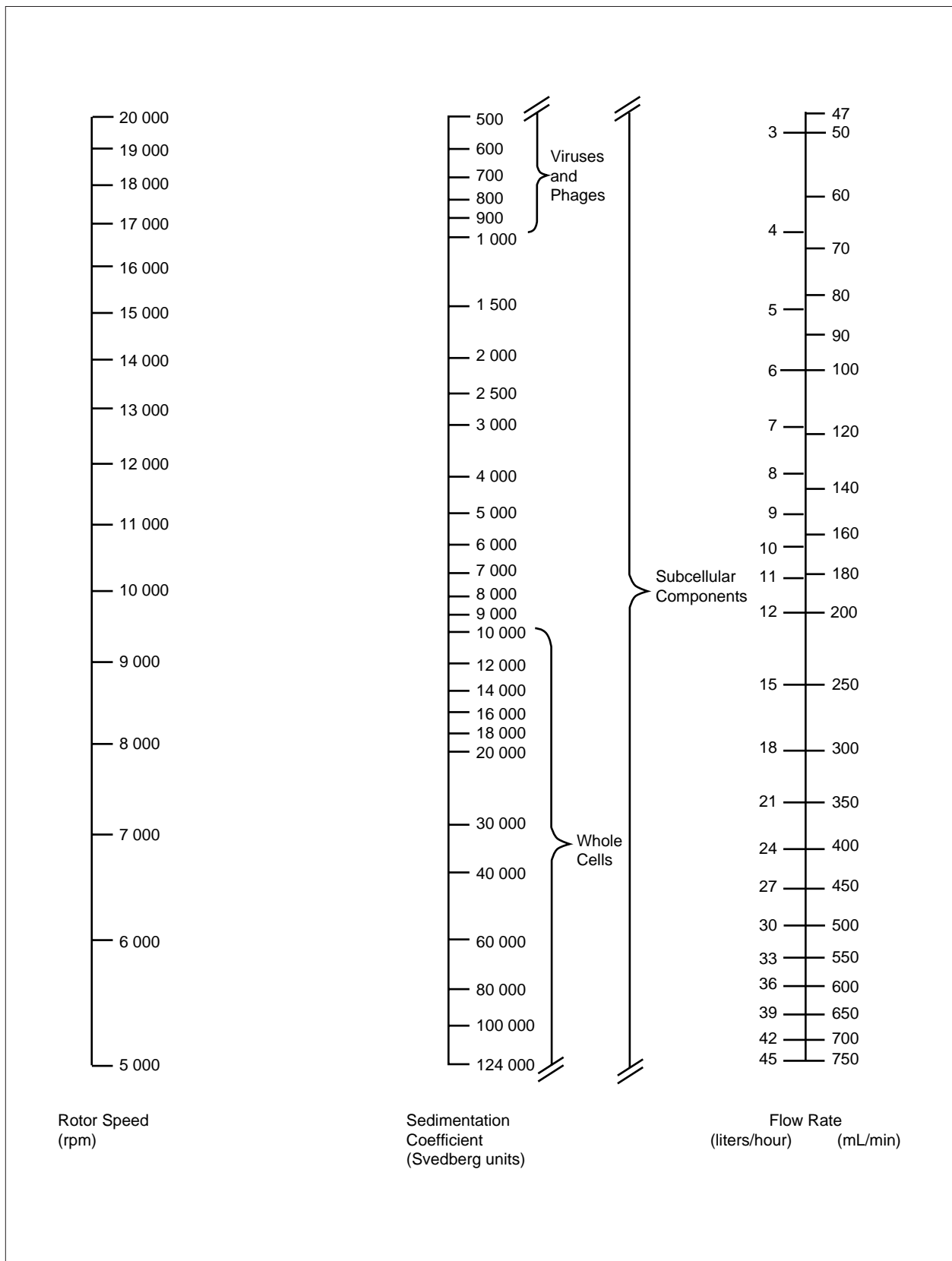
If high-risk agents are being investigated, the use of a Class III biological safety cabinet with a modified centrifuge should be considered. Beckman Coulter will be pleased to work with you on the design of an appropriate system.

Q The solutions I need to use are harmful to the Noryl material in the core. Is there an alternative?

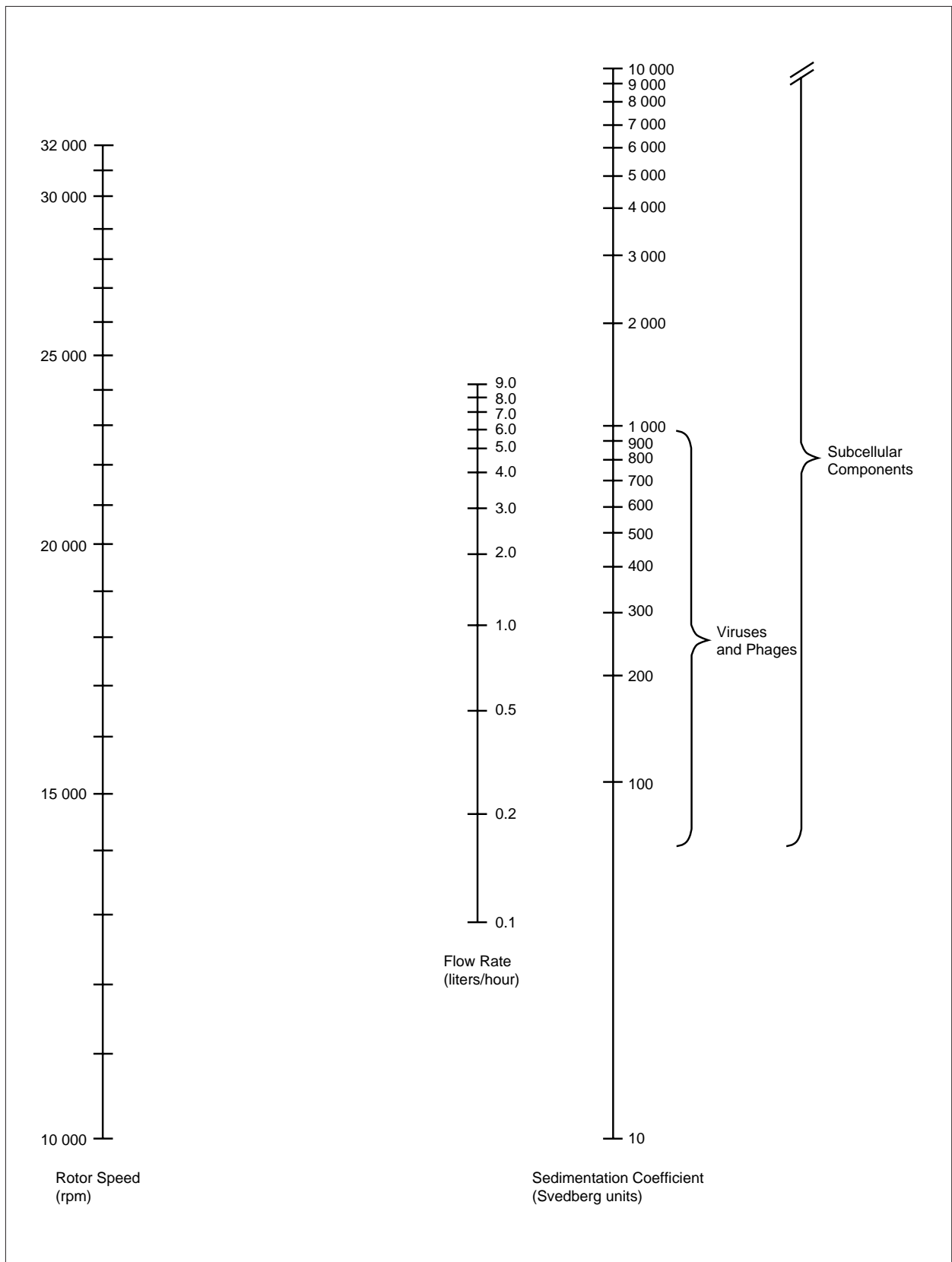
A Upon specific request, the continuous flow cores can be manufactured from titanium. Contact the CDC Marketing Department for further information.

Q What should I do if I am unsure of the condition of my continuous flow rotor?

A Beckman Coulter, Inc., will provide free inspection of any rotor at the request of the user. Rotor owners are urged to make use of this service if they have any doubts about the condition of a rotor.



JCF-Z rotor nomogram. Theoretical maximum flow rate for 100% cleanout when using Standard Core. To use, place a ruler on the page to intersect the middle column (known Svedberg units). Pivot the ruler about this point to intersect the other two columns. The nomogram covers all practical combinations of speed and flow rate.



CF-32 Ti rotor nomogram. Theoretical maximum flow rate for 100% cleanout. To use, place a ruler on the page to intersect the right-hand column (known Svedberg units). Pivot the ruler about this point to intersect the other two columns. The nomogram covers all practical combinations of speed and flow rate.

Bibliography of Applications

References to some typical uses of the CF-32 Ti and JCF-Z continuous flow rotors are given below. (Some of the earlier literature mentions the CF-35 Ti rotor. This rotor is now designated the CF-32 Ti, and operates at a

maximum speed of 32,000 rpm.)

The references are grouped according to the material separated; the rotor used and the type of separation are given for each one.

Algae

Chlamydomonas reinhardtii JCF-Z, pelleted.

Schleicher, M., Lukas, T. J., Watterson, D. M. Isolation and characterization of calmodulin from the motile green alga *Chlamydomonas reinhardtii*. *Arch. Biochem. Biophys.* 229, 33-42 (1984)

Synechococcus cedrorum JCF-Z, pelleted.

Newman, P. J., Sherman, L. A. Isolation and characterization of photosystem I and II membrane particles from the blue-green algae, *Synechococcus cedrorum*. *Biochim. Biophys. Acta* 503, 343-361 (1978)

Bacteria

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Eriksson, P.-O., Rilfors, L., Wieslander, A., Lundberg, A., Lindblom, G. Order and dynamics in mixtures of membrane glucolipids from *Acholeplasma laidlawii* studied by ²H NMR. *Biochemistry* 30, 4916-4924 (1991)

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Escherichia coli JCF-Z, pelleted

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Scherzer, E., Auer, B., Schweiger, M. Identification, purification, and characterization of *Escherichia coli* virus T1 DNA methyl-transferase. *J. Biol. Chem.* 262, 15225-15231 (1987)

Wagenknecht, T., Bloomfield, V. A. *In vitro* polymerization of bacteriophage T4D tail core subunits. *J. Mol. Biol.* 116, 347-359 (1977)

Zidwick, M. J., Keller, G., Rogers, P. Regulation and coupling of *argECBH* mRNA and enzyme synthesis in cell extracts of *Escherichia coli*. *J. Bacteriol.* 159, 640-646 (1984)

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Robertson, D. E., Kroon, P. A., Ho, C. Nuclear magnetic resonance and fluorescence studies of substrate-induced conformational changes of histidine-binding protein J of *Salmonella typhimurium*. *Biochemistry* 16, 1443-1451 (1977)

Spirochetes JCF-Z, pelleted.

Livermore, B. P., Johnson, R. C. Lipids of the *Spirochaetales*: comparison of the lipids of several members of the genera *Spirochaeta*, *Treponema*, and *Leptospira*. *J. Bacteriol.* 120, 1268-1273 (1974)

Staphylococcus aureus JCF-Z, pelleted.

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Streptococcus pyogenes JCF-Z, culture fluid clarified.

Cunningham, C. M., Barsumian, E. L., Watson, D. W. Further purification of group A streptococcal pyrogenic exotoxin and characterization of the purified toxin. *Infect. Immun.* 14, 767-775 (1976)

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Virus-infected Vero cells JCF-Z, pelleted.

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Cell Culture Media

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Uckert, W., Wunderlich, V., Bender, E., Sydow, G., Bierwolf, D. The protein pattern of PMF virus, a type-D retrovirus from malignant permanent human cell lines. *Arch. Virol.* 64, 155-166 (1980)

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Miscellaneous

Brain protein and protein precipitates

JCF-Z, clarified, pelleted.

Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bolton, D. C., Bendheim, P. E., Groth, D. F., Glenner, G. G. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* 35, 349-358 (1983)

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Turk, E., Teplow, D. B., Hood, L. E., Prusiner, S. B. Purification and properties of the cellular and scrapie hamster prion proteins. *Eur. J. Biochem.* 176, 21-30 (1988)

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Reid, P. M., Wilkinson, A. E., Tipping, E., Jones, M. N. Determination of molecular weights of humic substances by analytical (UV scanning) ultracentrifugation. *Geochim. Cosmochim. Acta* 54, 131-138 (1990)

Subcellular Fractions

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Lysate, Human platelet JCF-Z, supernatant clarified.

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Cacace, M. G., Nucci, R., Reckert, H. Large scale preparation of calf liver nuclei by continuous flow centrifugation. *Experientia* 33, 855-857 (1977)

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Rosen, P., Ehrlich, B., Junger, E., Bubenzer, H. J., Kühn, L. Binding and degradation of insulin by plasma membranes from bovine liver isolated by a large scale preparation. *Biochim. Biophys. Acta* 587, 593-605 (1979)

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Hertzberg, E. L. Isolation and characterization of liver gap junctions. *Methods in Enzymology*, Vol. 98, pp. 501-510. Edited by S. Fleischer and B. Fleischer. New York, Academic Press, 1983.

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Goodenough, D. A. Bulk isolation of mouse hepatocyte gap junctions. Characterization of the principal protein, connexin. *J. Cell Biol.* 61, 557-563 (1974)

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Sharpee, R. I., Mebus, C. A., Bass, E. P. Characterization of a calf diarrheal coronavirus. *Am. J. Vet. Res.* 37, 1031-1041 (1976)

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Yeast

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