BECKMAN

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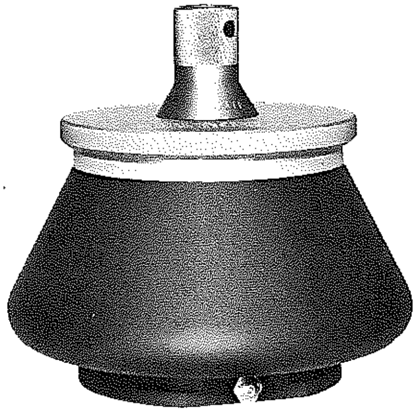
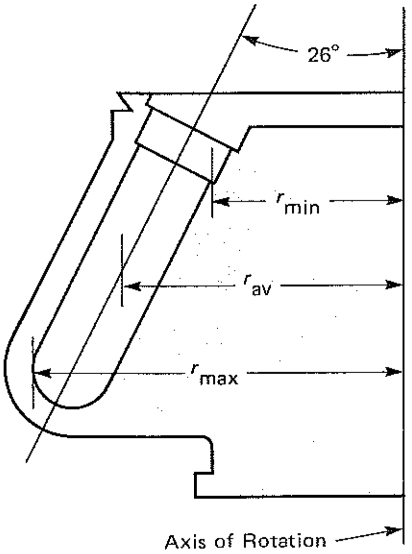
INSTRUCTIONS FOR USING THE TYPE 50 Ti ROTOR
In Beckman Class B, C, D, F, G, H, Q, and R Preparative Ultracentrifuges





SPECIFICATIONS

Maximum speed	50 000 rpm
Density rating at full speed	1.2 g/mL
Relative Centrifugal Field* at maxmum speed	
At r_{max} (80.8 mm)	226 000 x g
At r_{av} (59.1 mm)	165 000 x g
At r_{min} (37.4 mm)	105 000 x g
k factor at maximum speed	78
Number of tube cavities	12
Available tubes	see Tables 1 and 2
Nominal dimensions of largest tube	$^5/_8$ x 3 in. (16 x 76 mm)
Nominal tube capacity	13.5 mL
Nominal rotor capacity	162 mL
Approximate acceleration time to maximum speed (rotor fully loaded) in an L8M ultracentrifuge	$5^1/_2$ min
Approximate deceleration time from maximum speed (rotor fully loaded) in an L8M ultracentrifuge	$4^1/_2$ min
Weight of fully loaded rotor	7 kg (15.5 lb)
Rotor material	titanium
Conditions requiring speed reduction	see Run Speeds

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* Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed ($r\omega^2$) to the standard acceleration of gravity (g) according to the following formula:

$$\mathrm{RCF} = \frac{r\omega^2}{g}$$

where r is the radius in millimeters, ω is the angular velocity in radians per second (2πRPM/60), and g is the standard acceleration of gravity (9807mm/s^2). After substitution:

$$\mathrm{RCF} = 1.12\ r \left(\frac{\mathrm{RPM}}{1000}\right)^2$$

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DESCRIPTION

The Type 50 Ti titanium fixed angle rotor, rated for 50 000 rpm in Beckman class B, C, D, F, G, H, Q, and R preparative ultracentrifuges, holds up to twelve 5/8-in diameter tubes at a 26-degree angle from the axis of rotation. This general purpose rotor is useful for many applications: concentrating proteins and subcellular particles and isopycnic banding of DNA. Up to 162 mL of gradient and sample can be centrifuged per run.

The exterior surface of the rotor is finished with black urethane paint for optimal temperature control. The aluminum handle and lid are red-anodized for corrosion resistance. The O-rings in the rotor handle and body are made of Buna N and maintain atmospheric pressure in the rotor during centrifugation. This rotor is protected from exceeding 50 000 rpm in ultracentrifuges with the photoelectric or the mechanical overspeed detection systems; it is equipped with an overspeed disk (see the Supply List) and two mechanical cartridges for this purpose. The Type 50 Ti rotor is warranted at maximum speed for 5000 runs, 10 000 hours of centrifugation, or 5 years, whichever occurs first (see the Warranty).

OPERATION

NOTE: Specific information about the Type 50 Ti rotor is given here. Information common to this and other rotors is contained in the Rotors and Tubes Manual, LR-IM, which should be used together with this bulletin for complete rotor and accessory operation.

TUBES AND BOTTLES

Tubes and bottles available for the Type 50 Ti rotor are detailed in Tables 1 and 2. Be sure to observe the maximum speed limits shown, and use *only* the tubes and bottles listed.

Temperature Limits

Polyallomer and polycarbonate containers should not be centrifuged below 2°C. Do not freeze polyallomer tubes before centrifugation, as they may become brittle and crack. Polyallomer and polycarbonate containers may be centrifuged at temperatures above 20°C, but they should be pretested under anticipated run conditions. Ultra-Clear™ tubes have been tested for use at temperatures between 4 and 20°C. For centrifugation at other temperatures, pretest these tubes as well. Stainless steel tubes can be centrifuged at any temperature.

Tube Preparation

Thinwall polyallomer and Ultra-Clear tubes must be filled as full as possible and capped to prevent tube collapse during centrifugation. Thickwall plastic tubes may be used with or without tube caps. Do not overfill capless tubes. Stainless steel tubes can be filled to any level, but all opposing tubes for a run must be filled to the same level.

Table 1. Available $^{5}/_{8}$-in. (16-mm) Diameter Tubes for the Type 50 Ti Rotor*

Description	Part Number	Required Accessory		Nominal Fill Volume (mL)	Maximum Speed (rpm)
		Description	Part Number		
Thinwall					
Ultra-Clear	344085	aluminum cap	330860	13.5	50 000
polyallomer	326814	aluminum cap	330860	13.5	50 000
Quick-Seal					
Ultra-Clear	344322	spacer	342696	13.5	50 000
polyallomer	342413	spacer	342696	13.5	50 000
polyallomer	344622	spacer	344634	10	50 000
polyallomer	345830	floating spacer	345828†	6.3	50 000
polyallomer	346562	floating spacer	345828†	4.2	50 000
Thickwall					
polyallomer	355640	none	none	8†	30 000
		aluminum cap	338907	13.5	50 000
polycarbonate	355630	none	none	8†	50 000
		aluminum cap	338907	13.5	50 000
Polycarbonate bottle	355603 (assembly) 355651 (bottle only)	Noryl cap	355604	10.4‡	50 000
Stainless Steel	301108	aluminum cap	303319	13.5	**

* All tubes are $^{5}/_{8}$ x 3 in (16 x 76 mm) except Quick-Seal tubes 344622 ($^{5}/_{8}$ x 2 $^{5}/_{8}$ in.; 16 x 67 mm), 345830 ($^{5}/_{8}$ x 1$^{3}/_{4}$ in.; 16 x 45 mm) and 356562 ($^{5}/_{8}$ x 1$^{1}/_{2}$ in.; 16 x 38 mm). *k* factors for these shorter tubes are 72, 50 and 44, respectively.

† Floating spacers, part of the g-Max system of tube support, are made of Noryl, a registered trademark of General Electric.

‡ Minimum fill volume is 5 mL.

** Read the section on Run Speeds before using stainless steel (SS) tubes.

Table 2. Small Open-Topped Tubes Used with Delrin Adapters for the Type 50 Ti Rotor

Tube				Required Adapter	Maximum Speed and RCF	*k* Factor
Dimensions and Volume	Description	Part Number	Required Cap			
$^{1}/_{2}$ x 2$^{1}/_{2}$ in (6.5 mL)	Ultra-Clear	344088	346256*	303313	40 000 rpm 133 000 x *g*	120
	polyallomer	326820	346256*			
	stainless steel	301099	305022			
$^{1}/_{2}$ x 2$^{1}/_{2}$ in (4 mL)	thickwall polycarbonate	355645	none			
	thickwall polyallomer	355644	none			
$^{1}/_{2}$ x 1$^{5}/_{8}$ in (4 mL)	Ultra-Clear	344093	346256*	303402	43 000 rpm 134 000 x *g*	69
$^{1}/_{2}$ x 1$^{1}/_{4}$ in (3 mL)	Ultra-Clear	344092	346256*	303401	43 000 rpm 126 000 x *g*	71
$^{5}/_{16}$ x 1$^{15}/_{16}$ (2 mL)	Ultra-Clear	344091	303624	303376	40 000 rpm 136 000 x *g*	69

*Includes the 344672 neoprene gasket and the 346246 stem.

Use only those caps listed in the tables. Do not use any substitutes. Cap O-rings and gaskets should be dry and free from lubricant during assembly. Aluminum or stainless steel caps should be tightened using a hex driver while the tube is held in the tube vise. (Refer to publication L5-TB-060.)

Polycarbonate bottles may be centrifuged completely filled, or if desired, partially filled with at least 5 mL of liquid. Use only caps listed in the table. Tighten caps by hand. Use the 335381 tool to remove bottles from the rotor.

Quick-Seal® tubes should be filled leaving a small air space at the neck of the tube. Do not leave a large air space. Too much air can cause the tube to deform, which may disrupt gradients or sample. See publications IN-163 and IN-18 for detailed information on the use and care of Quick-Seal tubes.

The *g*-Max™ system uses a combination of short bell-top Quick-Seal tubes (see Table 1) and floating spacers (also referred to as *g*-Max spacers). This provides an advantage over using conventional sleeve-type adapters, in which the tubes are no longer at the maximum radius, resulting in a reduction of *g* force. In the *g*-Max system, floating spacers sit on top of the Quick-Seal tubes–thus, there is no reduction of radial distance and, therefore, no reduction of *g* force. Further, adapters cannot be run at maximum speed. Finally, the shorter pathlength of the tubes permits faster run times for gradient or pelleting separations. For more information on the *g*-Max system, see publication DS-709.

ROTOR PREPARATION

Before using the rotor, be sure it is equipped with the correct overspeed protection device. For instrument classes G, H, and R use the 36-sector overspeed disk. For all other classes, the mechanical cartridges (see the Supply List for part numbers) should be installed in the sides of the rotor base. Be sure the threads in the rotor handle and body are well lubricated with Spinkote™ lubricant, and the O-rings are thinly coated with silicone vacuum grease. For runs at temperatures other than room temperature, always refrigerate or warm the rotor beforehand, since titanium is a poor conductor of heat. Tubes placed opposite each other in the rotor cavities should be filled to the same level with the same density liquid. Load the rotor symmetrically.

In Models L2-50/65 ultracentrifuges, use the stablizer level "50" for the Type 50 Ti rotor. In Models L2-65B/75B, use the fourth level (four dots).

RUN TIMES

The *k* factor of the rotor is a measure of the rotor's pelleting efficiency. (Beckman has calculated *k* factors for all of its preparative rotors at maximum rated speed and using full tubes.) The *k* factor is calculated from the formula:

$$k = \frac{\ln (r_{\max}/r_{\min})}{\omega^2} \times \frac{10^{-10}}{3.6} \qquad (1)$$

where ω is the angular velocity of the rotor in radians per second (ω = 0.105 x RPM), $r_{\max}$ is the maximum radius, and $r_{\min}$ is the minimum radius.

Use the k factor in the following equation to estimate the run time t (in hours) required to pellet particles of known sedimentation coefficient s (in Svedberg units).

$$t = \frac{k}{s} \qquad (2)$$

Run times can be estimated for centrifugation at less than maximum speed by adjusting the k factor as follows:

$$k_{\text{adj}} = k \left(\frac{\text{rated speed of rotor}}{\text{actual run speed}} \right)^2 \qquad (3)$$

Run times can also be estimated from data established in prior experiments if the k factor of the previous rotor is known. For any two rotors[1] a and b:

$$\frac{t_{\text{a}}}{t_{\text{b}}} = \frac{k_{\text{a}}}{k_{\text{b}}} \qquad (4)$$

where the k factors have been adjusted for the actual run speed used.

Finally, use equation (1) above to calculate the k factor when the column of liquid is such that the operational $r_{\max}$ and $r_{\min}$ are significantly different from the $r_{\max}$ and $r_{\min}$ of the rotor (e.g., when using partially filled tubes or adapters). For more information on k factors see the Rotors and Tubes Manual and publication DS-719.

RUN SPEEDS

The centrifugal force at a given radius in a rotor is a function of the rotor speed. Comparisons of forces between different rotors are made by comparing the rotors' relative centrifugal fields (RCF). When rotational speed is selected so that identical samples are subjected to the same RCF in two different rotors, one may then describe the samples as having been subjected to the same centrifugal force field (refer to Table 3).

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[1] The method is most accurate when comparing two like rotors, i.e., two vertical tube rotors or two fixed angle rotors.

Table 3. Relative Centrifugal Fields. Entries in this table are calculated from the formula RCR = 1.12 r (RPM/1000)² and then rounded to three significant digits.

Rotor Speed (rpm)	Relative Centrifugal Field (x *g*)			*k* Factor*
	At r_{max} (80.8 mm)	At r_{av} (59.1 mm)	At r_{min} (37.4 mm)	
50 000	226 000	165 000	105 000	78
46 000	191 000	140 000	88 600	92
45 000	183 000	134 000	84 800	96
43 000	167 000	122 000	77 500	106
40 000	145 000	106 000	67 000	122
37 500	127 000	93 100	58 900	139
35 000	111 000	81 100	51 300	159
31 500	89 800	65 700	41 600	197
30 000	81 400	59 600	37 700	217
25 000	56 600	41 400	26 200	312
20 000	36 200	26 500	16 800	488

* Calculated for all Beckman preparative rotors as a measure of the rotor's relative pelleting efficiency in water at 20°C.

Rotor speeds may not be selected in excess of those provided in Tables 1 and 2 for particular tubes and caps. In addition, rotor speeds must be reduced in the following circumstances.

1. For centrifuging nonprecipitating solutions of density greater than 1.2 g/mL in *plastic tubes*, use the following square-root reduction formula to determine the allowable rotor speed.

$$\text{reduced maximum speed} = 50\,000\ \text{rpm}\sqrt{\frac{1.2\ \text{g/mL}}{\text{density of tube contents}}} \qquad (5)$$

   This speed reduction will protect the rotor from excessive stresses due to the added load.

2. When CsCl or other self-forming gradient salt is centrifuged, the square-root reduction formula above usually will not guard against the precipitation of salt crystals. Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation will alter the density distribution, and therefore sample separation. Figures 1 and 2, together with the description and examples, show how to reduce rotor speed when using CsCl gradients.

3. When centrifuging *stainless steel tubes*, the maximum permissible rotor speed depends on the type of cap assembly used and the total load to be centrifuged.

a. Maximum speeds based on the type of cap assembly are:
   for all-aluminum caps: 50 000 rpm
   for aluminum caps with stainless steel stems: 37 500 rpm

b. Maximum speeds based on the tube load should be determined from the equation:

$$\text{reduced maximum speed} = 50\,000\ \text{rpm}\sqrt{\frac{16.2\ \text{grams}}{M}} \tag{6}$$

where M is the mass in grams of the capped stainless steel tube plus the liquid contents to be centrifuged. *NOTE: In all cases, the lower speed determined in a and b is the limiting speed.*

SELECTING CsCl GRADIENTS

Rotor speed is used to control the shape of a CsCl gradient, but precipitation of CsCl during centrifugation must be prevented. Speed and density combinations that intersect on or below the curves in Figure 1 ensure that CsCl will not precipitate during centrifugation of the Type 50 Ti rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). Note that for a given initial homogeneous CsCl solution density, the maximum allowable speed increases as the fill volume of solution in the tube decreases.

The curves in Figure 2 show gradient profiles at equilibrium. Each curve was generated for a single rotor speed using the maximum allowable homogeneous CsCl densities (one for each fill volume) that avoid precipitation at that speed and temperature.[2] Figure 2 can be used to approximate the banding positions of sample particles. In general, lower speeds provide better particle resolution, but will require longer run times to achieve particle equilibration.

> **NOTE:** The curves in Figures 1 and 2 are for solutions of CsCl salt only. If other salts are present in significant concentrations, the overall CsCl concentration or the rotor speed must be reduced. This prevents precipitation of salts concentrated at the tube bottom.

Solutions can be centrifuged faster (therefore for shorter run times) when a tube is partially filled with gradient and sample. (For centrifuging partially filled thinwall tubes, fill the remainder of the tube with a low-density, immiscible liquid, such as mineral oil. (Do not use an oil overlay in Ultra-Clear tubes.) For example, a *full* tube of a 1.65 g/mL homogeneous CsCl solution can be centrifuged no faster than 40 000 rpm at 20°C (from Figure 1). Figure 2 presents the gradient profile, from 1.485 g/mL at the meniscus to 1.86 g/mL at the tube bottom. The same solution in a 3/4-filled tube can be centrifuged at 20°C at 44 000 rpm.

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[2] The gradients in Figure 2 can be generated from step or linear gradients, or from homogeneous solutions.

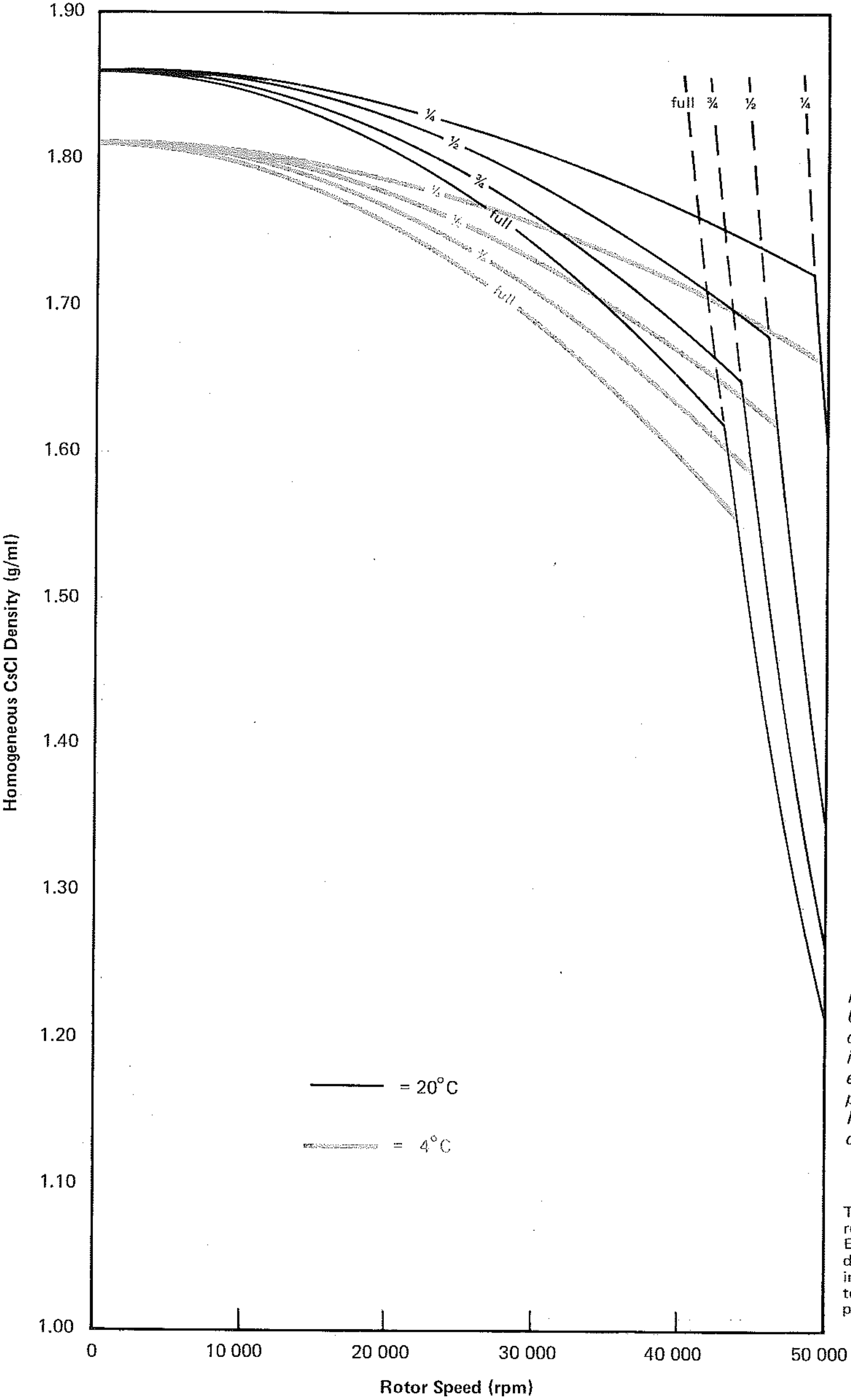
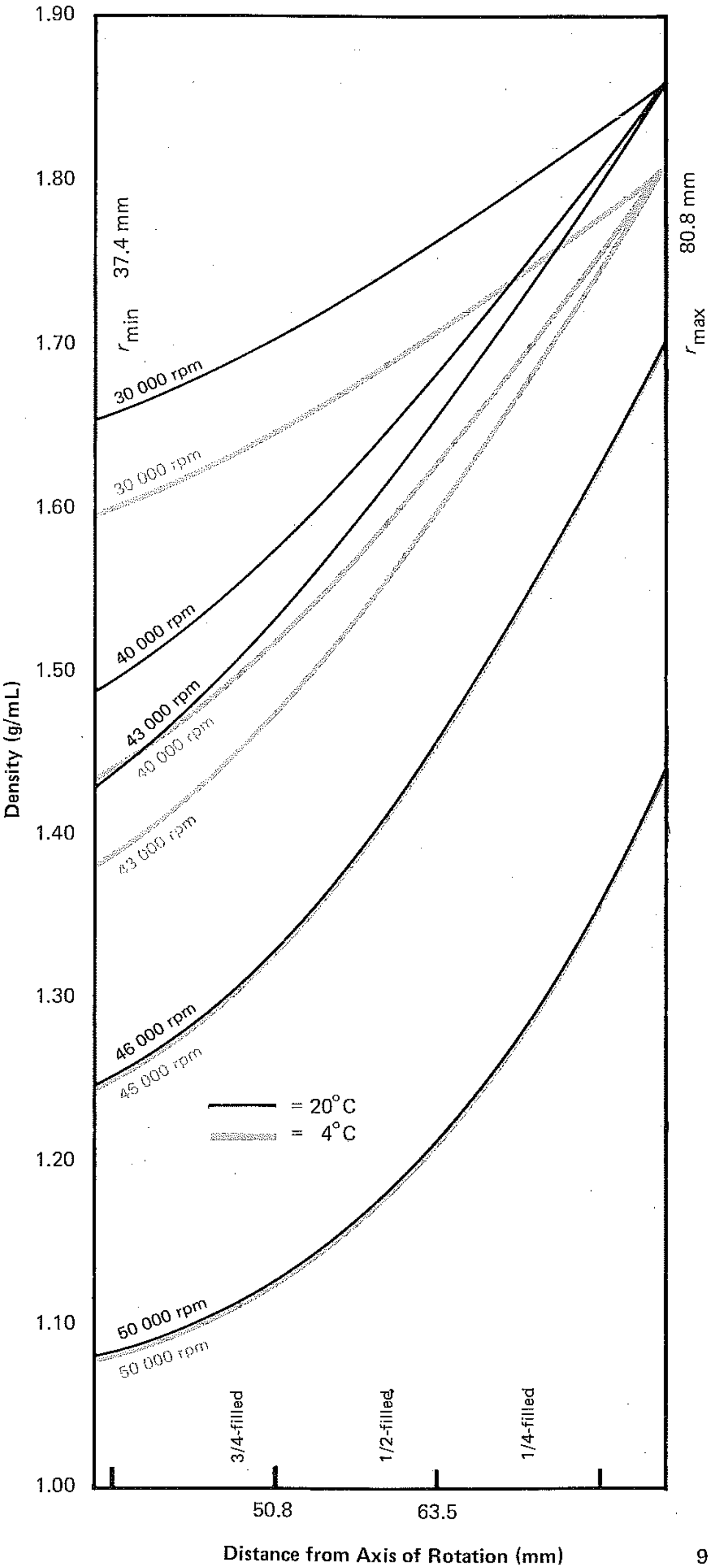


Figure 1. Precipitation Curves. Using combinations of CsCl densities and rotor speeds that intersect on or below the curves ensures that CsCl will not precipitate during centrifugation. Fill volumes are indicated on the curves.

The dashed lines are a representation of Equation (4) and are drawn here to show the inability of that formula to guard against CsCl precipitation.

Figure 2. CsCl Gradients at Equilibrium. Centrifugation of homogeneous CsCl solutions at maximum allowable speeds (from Figure 1) results in the gradients presented. Density increases from the top to the bottom of the tube (from 37.4 to 80.8 mm from the axis of rotation).

Each square on the grid represents 0.010 g/mL by 1.27 mm.



Interpolation of Figure 2 between the 43 000 and 46 000 rpm curves to the 3/4-filled level gives the 44 000 rpm curve. The same solution in a 1/2-filled tube can be centrifuged at 46 500 rpm, and in a 1/4-filled tube at 50 000 rpm. Note that the 46 000 rpm and 50 000 rpm curves in Figure 2 are identical for 4°C and 20°C. The 1/4-filled volume at 50 000 rpm yields a gradient distribution of 1.28 to 1.44 g/mL at the tube bottom.

TYPICAL EXAMPLES OF DETERMINING RUN PARAMETERS

Example A: Starting with a homogeneous CsCl solution density (e.g., 1.50 g/mL) and approximate particle densities (e.g., 1.48 and 1.52 g/mL), where will particles band?

1. In Figure 1 find the curve that corresponds to the desired run temperature (20°C) and tube fill volume (3/4-filled). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (46 000 rpm).
2. In Figure 2, sketch a horizontal line corresponding to each particle density.
3. Mark the point where each density intersects the curve corresponding to the maximum speed and selected temperature.
4. Particles will band at these points along the tube axis.

In this example, particles will band at about 65 and 68 mm from the axis of rotation (about 3 mm of interband separation at the 26-degree tube angle, or d_θ). When the tube is held upright, there will be about 7 mm of interband separation ($d_{up} = d_\theta / \sin\theta$). About 16 hours of centrifugation is required for particle equilibration. If a full tube is used instead of a 3/4-filled tube, the maximum allowable speed for a 1.50 g/mL solution is 45 000 rpm and about 20 hours of centrifugation will be required.

Example B: Knowing particle densities (e.g., 1.70 and 1.65 g/mL), how do you achieve good separation?

1. In Figure 2, sketch a horizontal line corresponding to each particle density.
2. Select the curve at the desired temperature (4°C) and tube volume (full) that gives good separation.
3. Note the speed indicated along the curve (30 000 rpm).
4. From Figure 1, determine the maximum allowable homogeneous CsCl density that corresponds to the selected run parameters (temperature, speed, and fill volume), 1.695 g/mL. These parameters will provide the particle separation selected in Step 2.

In this example, particles will band at about 50 and 61 mm from the axis of rotation (about 11 mm of interband separation at the tube angle). When the tube is held upright, there will be about 25 mm of interband separation.

MAINTENANCE

Routinely inspect the overspeed device. If the disk is scratched, damaged, or missing, replace it. If a mechanical cartridge has tripped, replace both cartridges, as the other has also fatigued. Instructions for installing a new device are given in the Rotors and Tubes Manual. Do not use sharp tools on the rotor. Store the rotor in a dry environment (not in the instrument) with the lid removed.

Silicone vacuum grease should be routinely applied to the O-rings in the handle and rotor body. Replace the O-rings every 6 months or whenever worn or damaged. Keep the rotor threads well lubricated with Spinkote lubricant. Refer to the Rotors and Tubes Manual for the chemical resistance of rotor and tube materials. Contact your Beckman Field Service Representative for information about the rotor repair program and the Field Rotor Inspection Program.

RETURNING A ROTOR

Before returning a rotor or accessory for any reason, prior permission (a Returned Goods Authorization form) must be obtained from Beckman Instruments. This RGA form may be obtained from your local Sales Office. It should contain the following information:

- serial number,
- a history of use (approximate frequency of use),
- the reason for the return,
- the original purchase order number, billing number, and shipping number, if possible,
- the name and phone number of the person to be notified upon receipt of the rotor or accessory at the factory, and
- the name and phone number of the person to be notified about repair costs, etc.

To protect our personnel, it is the customer's responsibility to ensure that the parts are free from pathogens and/or radioactivity. Sterilization and decontamination must be done before returning the parts. Smaller items (such as tubes, bottles, etc.) should be enclosed in a sealed plastic bag.

All parts must be accompanied by a note, plainly visible on the outside of the box or bag, stating that they are safe to handle and that they are not contaminated with pathogens or radioactivity. Failure to attach this notification will result in return or disposal of the items without review of the reported problem.

SUPPLY LIST

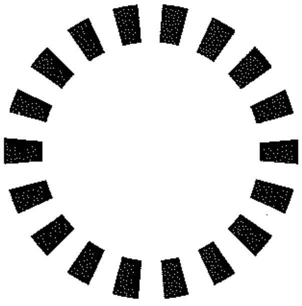
REPLACEMENT ROTOR SUPPLIES

See the Rotors, Tubes, and Accessories catalog (PL-174) for detailed information on reordering supplies. For your convenience, a partial list is given below.

Type 50 Ti Rotor	326221
Tubes, bottles, adapters, floating spacers and spacers	see Tables 1 and 2
Rotor handle	334968
Handle O-ring	011757
Rotor O-ring	807474
Overspeed disk (50 000 rpm)	330336
Mechanical overspeed dvice (50 000 rpm)	335158

OTHER SUPPLIES

Solution 555	339555
Rotor Cleaning Kit	339558
Silicone vacuum grease	335148
Spinkote lubricant	306812
Tube-sealing kit (with 60-Hz sealer)	342429
Tube-sealing kit (with 50-Hz sealer)	342424
Tube-sealer racks for Quick-Seal tubes:	
tubes 344322 and 342413	342488
tube 345830	345833
tube 344622	344641
Tube Topper kit (60 Hz)	348137
Tube Topper kit (50 Hz)	349647
Tube rack for 5/8-in. diameter tubes (24 places)	348123
Tube removal tool for capped tubes	301875
Tube-cap vise	305075
Hex driver for 7/16-in. (22 mm) cap nuts	841883
Hex driver for 5/16-in. (7.8 mm) cap nuts (for tube cap 303624)	841884
Tube removal tool for polycarbonate bottles with Noryl[4] caps	335381



36-sector Overspeed Disk (50 000 rpm)

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[4] Registered trademark of General Electric.