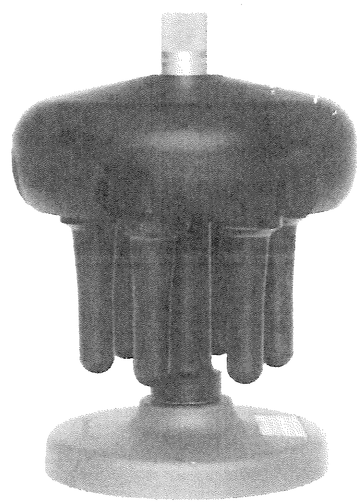
Instructions For Use

SW 41 Ti Swinging-Bucket Rotor

For Use in Beckman Coulter
Class H, R, and S
Preparative Ultracentrifuges



L5-TB-047QA
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Beckman Coulter, Inc.
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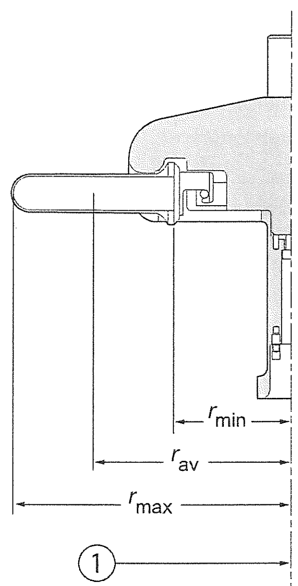
BECKMAN COULTER

SW 41 Ti
Swinging-Bucket Rotor

Specifications



1. Axis of Rotation

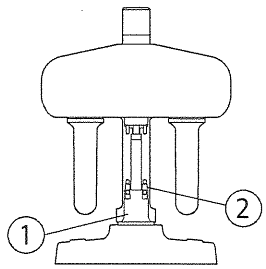
U.S. Pat. No. 3,393,864;
Japanese Pat. No. 739,613;
British Pat. No. 1,145,005;
German Pat. No. 1,598,174

Maximum speed	41,000 RPM
Density rating at maximum speed	1.2 g/mL
Relative Centrifugal Field[a] at maximum speed	
At r_{max} (153.1 mm)	288,000 × *g*
At r_{av} (110.2 mm)	207,000 × *g*
At r_{min} (67.4 mm)	127,000 × *g*
k factor at maximum speed	124
k factor at maximum speed (5 to 20% sucrose gradient; 5°C)	
When particle density = 1.3 g/mL	335
When particle density = 1.5 g/mL	307
When particle density = 1.7 g/mL	295
Conditions requiring speed reductions	see *Run Speeds*
Number of buckets	6
Available tubes	see Table 1
Nominal tube dimensions (largest tube)	14 × 89 mm
Nominal tube capacity (largest tube)	13.2 mL
Nominal rotor capacity	79.2 mL
Approximate acceleration time to maximum speed (fully loaded)[b]	7 min
Approximate deceleration time from maximum speed (fully loaded[b]	6 min
Weight of fully loaded rotor	6.4 kg (14 lb)
Rotor material	titanium

a. Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed (rw^2) to the standard acceleration of gravity (g) according to the following formula: RCF = $r\omega 2/g$ — where r is the radius in millimeters, ω is the angular velocity in radians per second (2π RPM /60), and g is the standard acceleration of gravity (9807 mm/s^2). After substitution: RCF = 1.12r (RPM/1000)^2

b. Time may vary depending on which instrument is used.

Description



1. Adapter
2. Drive Pins

This Beckman Coulter rotor has been manufactured in an ISO 9001 or 13485 facility for use with the specified Beckman Coulter ultracentrifuges.

The SW 41 Ti is a swinging bucket rotor designed to centrifuge up to six tubes. Used in Beckman Coulter class H, R, and S preparative ultracentrifuges, the rotor develops centrifugal forces for the separation and purification of small particles. Typical applications include separation of RNA, proteins, and subcellular particles in solution using rate zonal centrifugation. Approximate sample volume per tube is 0.5 mL, with a gradient volume of about 12.5 mL.

The rotor body and buckets are made of titanium and finished with black polyurethane paint. A solid-film lubricant (grey in color) is applied to the bucket flange to improve the seating of the bucket into the rotor pocket. Bucket caps are anodized aluminum. The bucket and cap assemblies hook over the crossbar of the rotor hanger mechanism. Gaskets, made of Buna N rubber, between each bucket and bucket cap maintain atmospheric pressure inside the buckets during centrifugation.

NOTE On some swinging bucket rotors a solid film lubricant coating is added to the bucket flange where the bucket contacts the rotor body. The purpose of the coating, which is a dull gray in color, is to minimize friction and enable the bucket to swing into the rotor bucket pocket more smoothly. With use and handling, all or part of this coating may wear off; this should not affect the rotor performance, as the bucket swing-up will wear in with use.

Drive pins in the rotor bottom prevent the rotor from slipping on the ultracentrifuge drive hub during acceleration and deceleration. Two indentations on the sides of the rotor adapter indicate their location.

For overspeed protection, a Beckman Coulter ultracentrifuge equipped with a photoelectric detector will monitor the overspeed disk on the adapter bottom and shut down the run if a speed exceeding the maximum allowable speed is detected.

See the Warranty at the back of this manual for warranty information.

Preparation and Use

Specific information about the SW 41 Ti rotor is given here. Information common to this and other rotors is contained in Rotors and Tubes for Preparative Ultracentrifuges (publication LR-IM), which should be used together with this manual for complete rotor and accessory operation. Publication LR-IM is included in the literature package with this rotor manual.

NOTE Although rotor components and accessories made by other manufacturers may fit in the SW 41 Ti rotor, their safety in this rotor cannot be ascertained by Beckman Coulter. Use of other manufacturers' components or accessories in the SW 41 Ti rotor may void the rotor warranty and should be prohibited by your laboratory safety officer. Only the components and accessories listed in this publication should be used in this rotor.

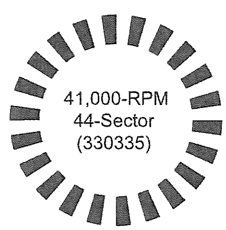
Prerun Safety Checks

Read the Safety Notice section at the front of this manual before using the rotor.

1 Make sure that the rotor, buckets, and caps are clean and show no signs of corrosion or cracking.

2 Make sure that the rotor is equipped with the correct overspeed disk.

  a. If the disk is missing or damaged, replace it according to the instructions in *Rotors and Tubes.*



3 Verify that only the tubes and accessories listed in Table 1 are being used.

4 Check the chemical compatibilities of all materials used.

- Refer to *Chemical Resistances* (publication IN-175), included in the *Rotors and Tubes* CD.

Rotor Preparation

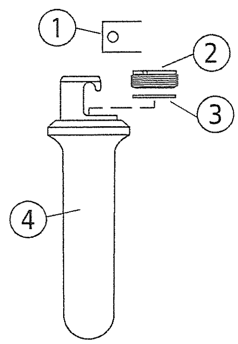
For runs at other than room temperature refrigerate or warm the rotor beforehand for fast equilibration.

1 Load the filled containers into the buckets (see page 6 for tube and accessory information).

a. Complete loading by placing the correct floating spacers (if required) over the tubes.

2 Ensure that bucket gaskets are lightly but evenly coated with silicone vacuum grease.

a. Do not run a bucket without a gasket, as the bucket will leak.



1. Hanger
2. Cap (331763)
3. Gasket (331309)
4. Bucket (333790)

3 Be sure that metal threads in the bucket caps are clean and lightly but evenly lubricated with Spinkote™ lubricant.

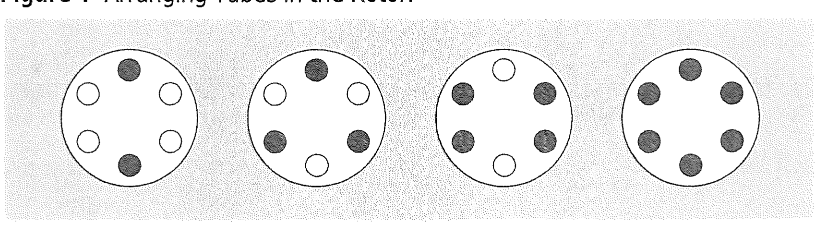
a. Put bucket caps on the buckets and use a screwdriver to screw the caps into the buckets until there is metal-to-metal contact.

4 *Hook all buckets, loaded or empty, to the rotor.*

a. If fewer than six tubes are being run, they must be arranged symmetrically in the rotor (see Figure 1).

b. Opposing tubes must be filled to the same level with liquid of the same density

Figure 1 Arranging Tubes in the Rotor.



NOTE Two, three, four, or six tubes can be centrifuged per run if they are arranged in the rotor as shown. All buckets must be attached to the rotor, whether loaded or empty.

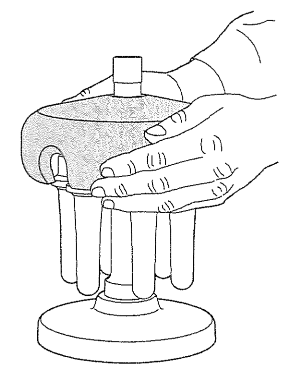
Operation

Refer to *Rotors and Tubes* for information on installing swinging bucket rotors.

1 To install the rotor, carefully lift it with both hands—*do not lift the rotor by the adapter*—and place it on the drive hub.

   a. Make sure that the rotor pins are perpendicular to the drive hub pins.

      - The pins must not rest on top of each other; turn the rotor to the right (clockwise) by hand to check for proper installation.



NOTE The aluminum handle supplied with the SW 41 Ti rotor is *not interchangeable* with similar handles supplied with other rotors.

2 Refer to the instrument instruction manual for ultracentrifuge operation.

3 For additional operating information, see the following:

- *Run Times*, page 9, for using *k* factors to adjust run durations.
- *Run Speeds*, page 9, for information about speed limitations.
- *Selecting CsCl Gradients*, page 11, for methods to avoid CsCl precipitation during centrifugation.

Removal and Sample Recovery

⚠ CAUTION

If disassembly reveals evidence of leakage, you should assume that some fluid escaped the rotor. Apply appropriate decontamination procedures to the centrifuge and accessories.

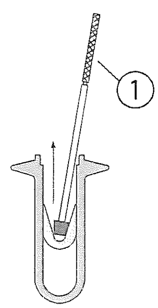
1 Remove the rotor from the centrifuge by lifting it straight up and off the drive hub.

2 Set the rotor on the rotor stand and carefully remove the buckets.

3 Remove the bucket caps and use the appropriate removal tool (listed in the *Supply List*) to remove the spacers and tubes.

a. If floating spacers were used, remove them with the threaded end of the floating spacer removal tool (338765).

NOTE If the conical-shaped adapters that support *k*onical tubes are difficult to remove after centrifugation, an extractor tool (354468) is available to facilitate removal.



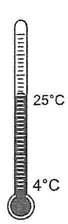
1. Extractor Tool (354468)

While pressing the rubber tip against the adapter wall, pull the tube and adapter up and out of the cavity.

Tubes and Accessories

The SW 41 Ti rotor uses tubes and accessories listed in Table 1. Be sure to use only those items listed, and to observe the maximum speed limits shown. Refer to Appendix A in *Rotors and Tubes* for information on the chemical resistances of tube and accessory materials.

Temperature Limits



- Plastic tubes have been centrifuge tested for use at temperatures between 4 and 25°C. For centrifugation at other temperatures, pretest tubes under anticipated run conditions.
- If plastic containers are frozen before use, make sure that they are thawed to at least 4°C prior to centrifugation.

Table 1 Available Tubes for the SW 41 Ti Rotor[a]

Tube			Required Accessory		Max Speed/ RCF/ *k* factor
Dimensions/ Nominal Volume/	Description	Part Number	Description	Part Number	
14 × 89 mm 13.2 mL	Ultra Clear	344059 (pkg/50)	none	—	41,000 RPM 288,000 × *g* 124
14 × 89 mm 13.2 mL	thinwall polyallomer	331372 (pkg/50)	none	—	41,000 RPM 288,000 × *g* 124
14 × 89 mm 10,2 mL	*k*onical open-top polyallomer	358120 (pkg/50)	adapter	358154	41,000 RPM 288,000 × *g* 124
14 × 89 mm 8.0 mL	Quick-Seal *k*onical, polyallomer	358649 (pkg/50)	adapter	358154	41,000 RPM 288,000 × *g* 108
			Noryl[b] floating spacer	355534	
14 × 47 mm 5.9 mL	Quick-Seal polyallomer	355537 (pkg/50)	Noryl floating spacer	355534	41,000 RPM 288,000 × *g* 55
14 × 48 mm 4.0 mL	Quick-Seal *k*onical, polyallomer	3358650 (pkg/50)	adapter	358154	41,000 RPM 288,000 × *g* 56
			Noryl floating spacer	355534	
14 × 25 mm 3.5 mL	Quick-Seal polyallomer	355870[c] (pkg/50)	Noryl floating spacer	355534	41,000 RPM 288,000 × *g* 27

a. Use only the items listed here..
b. Noryl is a registered trademark of GE Plastics.
c. Tube 55870 is also available in *g*-Max Kit 357330, which includes 50 tubes, six spacers (355534), and required tools.

Quick Seal Tubes

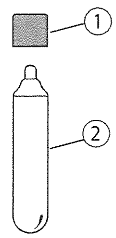
Quick-Seal® tubes must be sealed prior to centrifugation. These tubes are heat sealed and do not need caps; however, spacers are required on top of the tubes when they are loaded into the rotor buckets.

1 Fill Quick-Seal tubes leaving a *small* bubble of air at the base of the neck.

   a. Do not leave a large air space—too much air can cause excessive tube deformation.

2 Some of the Quick-Seal tubes listed in Table 1 are part of the *g*-Max™ system, which uses a combination of small bell-top Quick-Seal tubes and floating spacers (also called *g*-Max spacers).

- This means that you can run the shorter tubes listed in Table 1 in the SW 41 Ti rotor without reduction in *g* force.
- For detailed information on the *g*-Max system see publication DS-709.



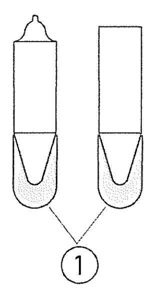
1. *g*-Max Spacer
2. Bell-top Tube

3 Refer to *Rotors and Tubes* for detailed information on the use and care of Quick-Seal tubes.

- Quick-Seal tubes are disposable and should be discarded after a single use.

*k*onical™ Tubes

Polyallomer *k*onical tubes, used to optimize pelleting separations, have a conical tip that concentrates the pellet in the narrow end of the tube. The narrow bottom also reduces the tube's nominal volume and minimizes gradient material requirement. The *k*onical tubes come in both open-top and Quick-Seal tube designs. Conical cavity adapters hold the tubes in the rotor buckets.



1. Adapters

Polyallomer and Ultra-Clear Open-Top Tubes

Polyallomer and Ultra-Clear® open-top tubes should be filled as full as possible (2 or 3 mm from the tube top) for tube support. If necessary, float mineral oil (or some other low-density, immiscible liquid) on top of the tube contents to fill the tube to its maximum volume. (Do not use an oil overlay in Ultra-Clear tubes.) All opposing tubes for a run must be filled to the same level with liquid of the same density.

Run Times

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

$$k = \frac{\ln(r_{max} / r_{min})}{\omega^2} \times \frac{10^{13}}{3600} \quad \textbf{EQ 1}$$

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

After substitution:

$$k = \frac{(2.533 \times 10^{11}) \ln(r_{max} / r_{min})}{\text{RPM}^2} \quad \textbf{EQ 2}$$

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, *S*).

$$t = \frac{k}{s} \quad \textbf{EQ 3}$$

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

$$k_{adj} = k \left(\frac{41,000}{\text{actual run speed}}\right)^2 \quad \textbf{EQ 4}$$

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, a and b:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \quad \textbf{EQ 5}$$

Run Speeds

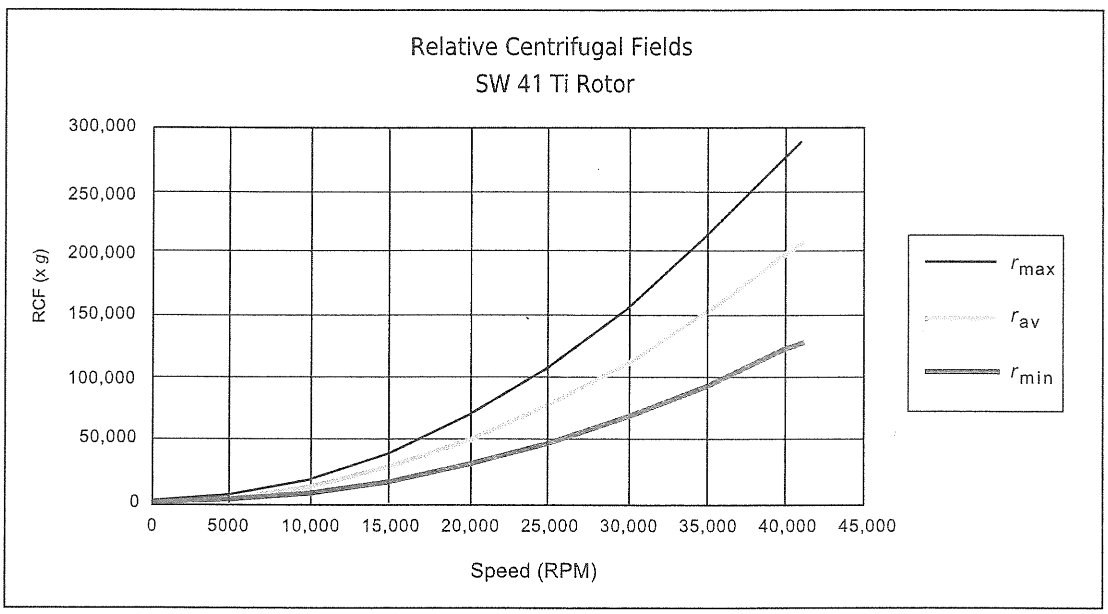
The centrifugal force at a given radius in a rotor is a function of speed. Comparisons of forces between different rotors are made by comparing the rotors' relative centrifugal fields (RCF). When rotational speed is adjusted so that identical samples are subjected to the same RCF in two different rotors, the samples are subjected to the same force. The RCF at a number of rotor speeds is provided in Table 2.

Table 2 Relative Centrifugal Fields for the SW 41 Ti Rotor[a]

Rotor Speed (RPM)	Relative Centrifugal Field (× *g*) At r_{max} (153.1 mm)	At r_{av} (110.2 mm)	At r_{min} (67.4 mm)	*k* Factor[b]
41,000	288,000	207,000	127,000	124
40,000	274,000	197,000	121,000	130
36,000	222,000	160,000	97,800	160
35,000	210,000	151,000	92,500	170
30,000	154,000	111,000	67,900	231
25,000	107,000	77,100	47,200	333
20,000	69,000	49,400	30,200	520
15,000	38,600	27,800	17,000	924
10,000	17,200	12,300	7,550	2078

a. Entries in this table are calculated from the formula RCF = 1.12r (RPM/1000)2 and then rounded to three significant digits.

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



Do not select rotational speeds in excess of 41,000 RPM. In addition, speeds must be reduced under the following circumstances:

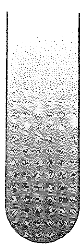
1. If nonprecipitating solutions more dense than 1.2 g/mL are centrifuged, the maximum allowable run speed must be reduced according to the following equation:

$$\text{reduced maximum speed} = (41{,}0000\ \text{RPM})\sqrt{\frac{1.2\ \text{g/mL}}{\rho}} \quad \textbf{EQ 6}$$

   where ρ is he density of the tube contents. This speed reduction will protect the rotor from excessive stresses due to the added tube load.

2. *Further speed limits must be imposed* when CsCl or other self-forming-gradient salts are centrifuged, as equation (6) does not predict concentration limits/speeds that are required to avoid precipitation of salt crystals. Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation may cause rotor failure. Figure 2 and Figure 3, together with the description and examples below, show how to reduce run speeds when using CsCl gradients.

Selecting CsCl Gradients



Rotor speed is used to control the slope of a CsCl density gradient, and must be limited so that CsCl precipitation is avoided. Speed and density combinations that intersect on or below the curves in Figure 3 ensure that CsCl will not precipitate during centrifugation in the SW 41 Ti rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). Curves in Figure 2 and Figure 3 are provided up to the maximum rated speed of the rotor.

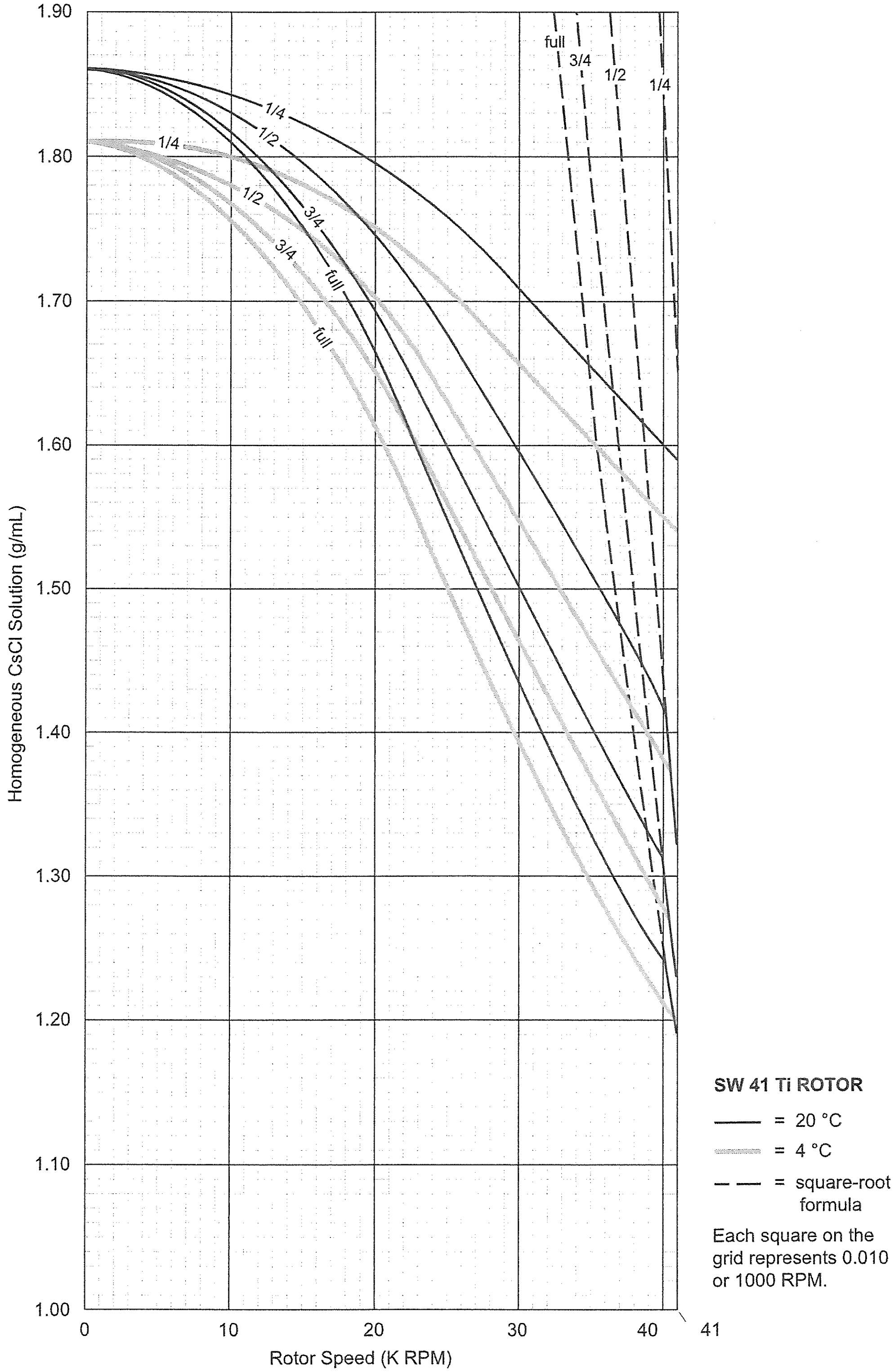
NOTE The curves in Figure 2 and Figure 3 are for solutions of CsCl salt dissolved in distilled water only. If other salts are present in significant concentrations, the overall CsCl concentration may need to be reduced.

The reference curves in Figure 3 show gradient distribution at equilibrium. Each curve in Figure 3 is within the density limits allowed for the SW 41 Ti rotor: each curve was generated for a single run speed using the maximum allowable homogeneous CsCl densities (one for each fill level) that avoid precipitation at that speed. (The gradients in Figure 3 can be generated from step or linear gradients, or from homogeneous solutions. But the total amount of CsCl in solution must be equivalent to a homogeneous solution corresponding to the concentrations specified in Figure 3.) Figure 3 can also be used to approximate the banding positions of sample particles. Curves not shown in the figure may be interpolated.

Adjusting Fill Volumes

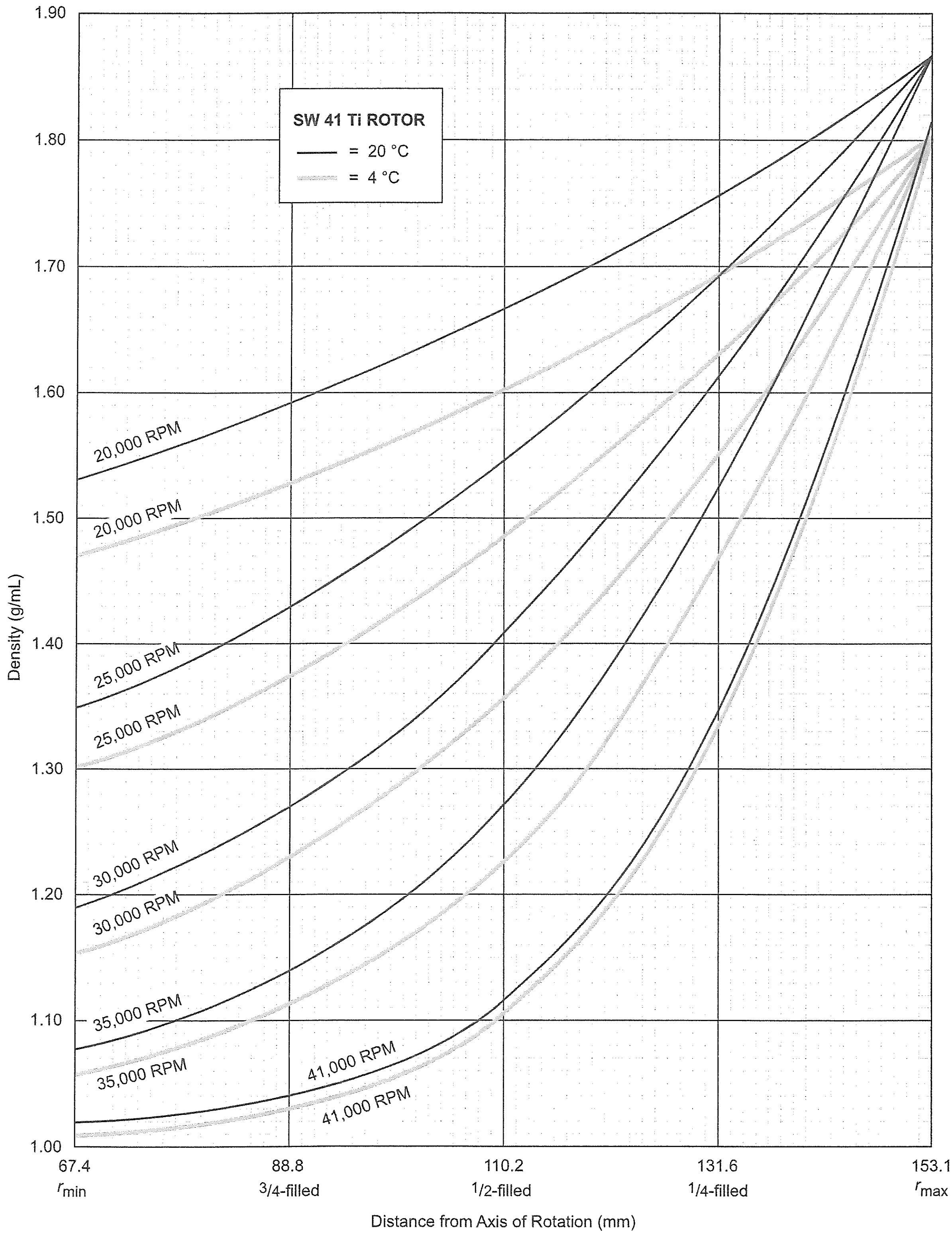
Figure 2 and Figure 3 show that several fill volumes are possible in a tube. If a thinwall tube is partially filled with gradient solution, float mineral oil (or some other low-density, immiscible liquid) on top of the tube contents to fill the tube to its maximum volume. (Do not use an oil overlay in Ultra-Clear tubes.) Note that for a given CsCl density, as the fill level decreases the maximum allowable speed increases. Partial filling may be desirable when there is little sample or when you wish to shorten the run time.

Figure 2 Precipitation Curves for the SW 41 Ti Rotor*



* Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation. The dashed lines are representations of equation (6), and are shown here to illustrate the inability of that equation to predict CsCl precipitation.

Figure 3 CsCl Gradients at Equilibrium for the SW 41 Ti Rotor*

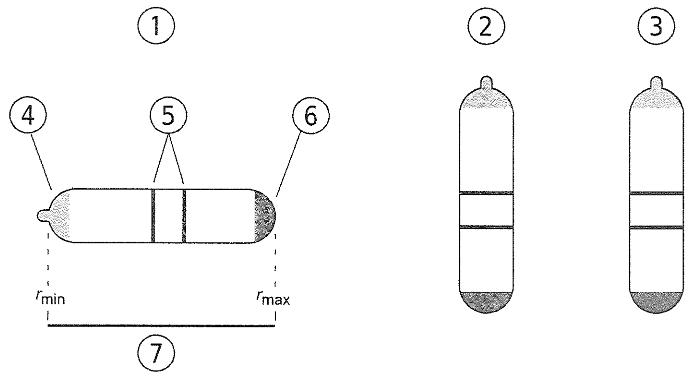


* Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 2) results in gradients presented here.

For example, a *quarter-filled* tube of 1.5-g/mL homogeneous CsCl solution at 4°C may be centrifuged at 41,000 RPM (see Figure 2). The segment of the 41,000-RPM curve (Figure 3) from the quarter-filled line to the tube bottom represents this gradient. The same solution in a *half-filled* tube may be centrifuged no faster than 33,000 RPM (curves not shown in the figure may be interpolated), and 28,000 RPM in a *three-quarter-filled* tube. A tube *full* of the 1.5-g/mL CsCl solution may be centrifuged no faster than 25,000 RPM.

Typical Examples for Determining CsCl Run Parameters

Example A:
Starting with a homogeneous CsCl solution density of 1.6 g/mL and approximate particle buoyant densities of 1.69 and 1.72 g/mL, at 20°C, where will particles band at equilibrium?



1. At Speed
2. At Rest in Rotor
3. At Rest Outside Rotor
4. Floating Components
5. Bands
6. Pelleted Material
7. Pathlength

1 In Figure 2 find the curve that corresponds to the required run temperature (20°C) and fill volume (one-half full).

- The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (30,000 RPM).

2 In Figure 3, sketch in a horizontal line corresponding to each particle's buoyant density.

3 Mark the point in the figure where each particle density intersects the curve corresponding to the selected run speed and temperature.

- Particles will band at these locations across the tube diameter at equilibrium during centrifugation.

In this example, particles will band about 138 and 141.5 mm from the axis of rotation, about 3.5 mm of centerband-to-centerband separation.

To determine interband volume in milliliters, use the following equation:

$$V = \pi r^2 h \quad \text{EQ 7}$$

where r is the tube radius in centimeters and h is the interband separation in centimeters

Example B:
Knowing particle buoyant densities (for example, 1.36 and 1.42 g/mL), how do you achieve good separation?

1 In Figure 3, sketch in a horizontal line corresponding to each particle's buoyant density.

2 Select the curve at the required temperature (4°C) and tube volume (full) that gives the best particle separation.

3 Note the run speed along the selected curve (25,000 RPM).

4 From Figure 2, select the maximum homogeneous CsCl density (in this case, 1.5 g/mL) that corresponds to the temperature and run speed established above.
   - These parameters will provide the particle-banding pattern selected in Step 2.

In this example, particles will band at about 85 and 97 mm from the axis of rotation (about 12 mm apart). The interband volume will be about 1.8 mL.

Care and Maintenance

Maintenance

NOTE Do not use sharp tools on the rotor that could cause scratches in the rotor surface. Corrosion begins in scratches and may open fissures in the rotor with continued use.

1 Regularly inspect the overspeed disk on the bottom of the rotor adapter.
   a. If it is scratched, damaged, or missing, replace it.
      - Replacement instructions are in *Rotors and Tubes.*

2 Frequently check the bucket gaskets (331309) for signs of wear.
   a. Replace gaskets every 6 months, or whenever worn or damaged.
   b. Keep the gaskets lightly coated with silicone vacuum grease.

3 Regularly lubricate the bucket cap threads with a thin, even coat of Spinkote lubricant (306812) before every run.

4 Refer to Appendix A in *Rotors and Tubes* for the chemical resistances of rotor and accessory materials.

- Your Beckman Coulter representative provides contact with the Field Rotor Inspection Program and the rotor repair center.

Cleaning

Wash the rotor and rotor components immediately if salts or other corrosive materials are used or if spillage has occurred. Do not allow corrosive materials to dry on the rotor.

Under normal use, wash the rotor frequently (at least weekly) to prevent buildup of residues.

1 Wash the rotor buckets, gaskets, and caps in a mild detergent, such as Beckman Solution 555™, that won't damage the rotor.

a. Dilute the detergent 10 to 1 with water.

NOTE Do not immerse the rotor body in water, since the hanger mechanism is difficult to dry and can rust.

- The Rotor Cleaning Kit contains two plastic-coated brushes and two quarts of Solution 555 (339555) for use with rotors and accessories.

2 Wash the rotor body with a sponge or cloth dampened with a mild detergent, such as Beckman Solution 555, diluted 10 to 1 with water.

3 Rinse the cleaned rotor and components with distilled water.

4 Air-dry the rotor and lid upside down.

a. *Do not use acetone to dry the rotor.*

5 Clean metal threads frequently to prevent buildup of residues and ensure adequate closure.

a. Use a brush and concentrated Solution 555.

b. Rinse and dry thoroughly, then lubricate lightly but evenly with Spinkote to coat all threads.

Supply List

NOTE Publications referenced in this manual can be obtained at www.beckmancoulter.com, by calling Beckman Coulter at 1-800-742-2345 in the United States, or by contacting your local Beckman Coulter office.

See the Beckman Coulter *Ultracentrifuge Rotors, Tubes & Accessories* catalog (BR-8101, available at www.beckmancoulter.com) or contact Beckman Coulter Sales (1-800-742-2345 in the United States) for detailed information on ordering parts and supplies. For your convenience, a partial list is given below.

Replacement Rotor Parts

Description	Part Number
SW 41 Ti rotor assembly	331362
Buckets (set of 6, with caps and gaskets)	333790
Bucket caps (set of 6)	331763
Bucket gasket	331309
Overspeed disk (41,000 RPM)	330335
Rotor stand	332400
Bucket holder rack	331313

Other

NOTE For MSDS information, go to the Beckman Coulter website at www.beckmancoulter.com.

Description	Part Number
Tubes and accessories	see Table 1
Quick-Seal Cordless Tube Topper kit, 60 Hz	358312
Quick-Seal Cordless Tube Topper kit, 50 Hz (Europe)	358313
Quick-Seal Cordless Tube Topper kit, 50 Hz (Great Britain)	358314
Quick-Seal Cordless Tube Topper kit, 50 Hz (Australia)	358315
Quick-Seal Cordless Tube Topper kit, 50 Hz (Canada)	367803
Tube Topper rack (14-mm dia. tubes)	356568
Floating spacer removal tool	338765
Tube removal tool (Quick-Seal tubes)	361668
Extractor tool (*k*onical tube adapters)	354468
Spinkote lubricant (2 oz)	306812

Description	Part Number
Silicone vacuum grease (1 oz)	335148
Rotor Cleaning Kit	339558
Beckman Solution 555 (1 qt)	339555
Rotor cleaning brush	339379
Centering tool (for overspeed disk replacement)	331325