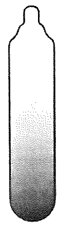
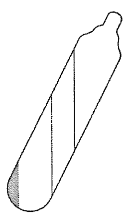
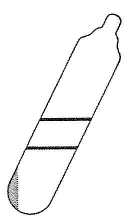
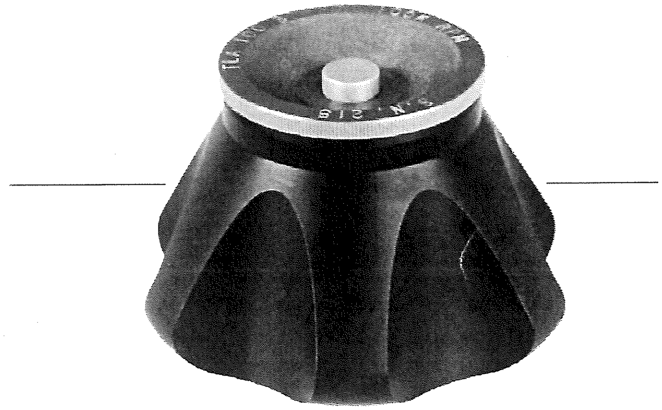
TL-TB-011AJ
September 2008

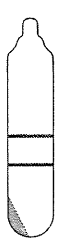


TLA-100.3 Rotor









Used In Beckman Coulter Optima™ MAX, MAX-XP, MAX-E, TL, TLX, and TL-100 Tabletop Ultracentrifuges

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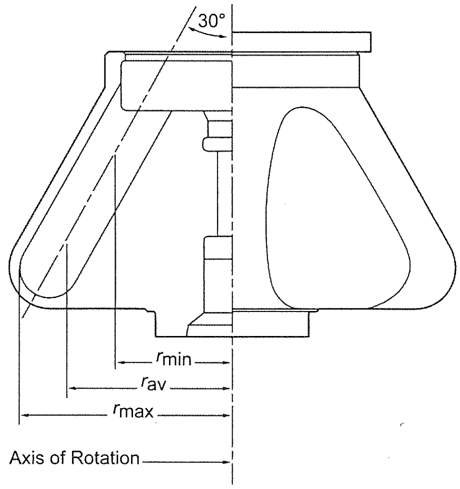
CA-0908-.2-FNG

TLA-100.3 ROTOR



U.S. Pat. No. 4,372,483
Japanese Pat. No. 1,551,443
Swiss Pat. No. 646,881

SPECIFICATIONS

Maximum speed	100 000 rpm
Density rating at maximum speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed†	
At r_{max} (48.3 mm)	541 000 × g
At r_{av} (37.9 mm)	424 000 × g
At r_{min} (27.5 mm)	308 000 × g
k factor at maximum speed	14
Conditions requiring speed reductions	see RUN SPEEDS
Number of tube cavities	6
Available tubes	see Table 1
Nominal tube dimensions (largest tube)	13 × 51 mm
Nominal tube capacity	3.5 mL
Nominal rotor capacity	21 mL
Approximate acceleration time to maximum speed (fully loaded)	4 min
Approximate deceleration time from maximum speed (fully loaded)	2 min
Weight of fully loaded rotor	1.0 kg (2.2 lb)
Rotor material	titanium

* 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:

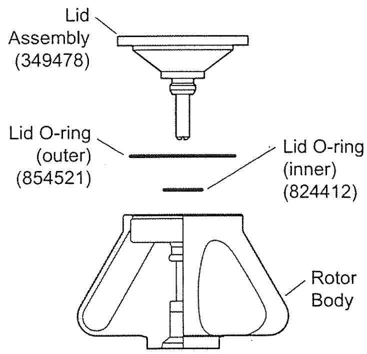
$$\text{RCF} = \frac{r\omega^2}{g}$$

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/s²). After substitution:

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

† 3.5-mL Quick-Seal® tubes

DESCRIPTION



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

The TLA-100.3 fixed angle rotor, rated for 100 000 rpm, has a tube angle of 30 degrees from the axis of rotation. The rotor can centrifuge up to six tubes and is used in Beckman Coulter Optima™ MAX, MAX-XP, MAX-E, TL, TLX, and the TL-100 tabletop ultracentrifuges.

The rotor is made of titanium and is finished with black polyurethane paint. The lid is made of aluminum and anodized to resist corrosion. A plunger in the lid locks the rotor to the drive hub before beginning the run, and two lubricated O-rings made of Buna-N rubber maintain atmospheric pressure inside the rotor during centrifugation. The tube cavities are numbered to aid in sample identification.

The rotor is specially designed with a fluid-containment annulus located below the O-ring sealing surface. The annulus retains fluid that may escape from leaking or overfilled tubes, thereby preventing the liquid from escaping into the instrument chamber.

The ultracentrifuge identifies rotor speed during the run by means of a magnetic speed sensor in the instrument chamber and magnets on the bottom of the rotor. This overspeed protection system ensures that the rotor does not exceed its maximum permitted speed.

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

PREPARATION AND USE

Specific information about the TLA-100.3 rotor is given here. Information common to this and other rotors is contained in Rotors and Tubes for Tabletop Preparative Ultracentrifuges *(publication TLR-IM), which should be used together with this manual for complete rotor and accessory operation. Publication TLR-IM is included in the literature package shipped with the rotor.*

NOTE

Although rotor components and accessories made by other manufacturers may fit in the TLA-100.3 rotor, their safety in this rotor cannot be ascertained by Beckman Coulter. Use of other manufacturers' components or accessories in the TLA-100.3 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

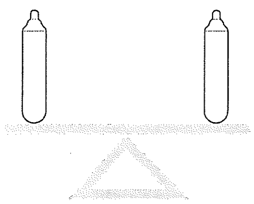
Read the Safety Notice page at the front of this manual before using the rotor.

1. Inspect the O-rings and plunger mechanism for damage—the high forces generated in this rotor can cause damaged components to fail.
2. Use only tubes and accessories listed in Table 1.
3. Check the chemical compatibilities of all materials used (refer to Appendix A in *Rotors and Tubes).*

ROTOR PREPARATION

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

1. Lightly but evenly lubricate metal threads with Spinkote™ lubricant (306812).
2. Apply a thin film of silicone vacuum grease (335148) to the two O-rings in the rotor lid.
3. If adapters are required, place them into the tube cavities before inserting the tubes.



4. Load the filled and capped tubes symmetrically into the rotor (see page 8 for tube information). If fewer than six tubes are being run, they must be arranged symmetrically in the rotor (see Figure 1). *Opposing tubes must be filled to the same level with liquid of the same density.*

5. Use the required spacers, if necessary (see Table 1), to complete the loading operation.

6. After the rotor is loaded, insert it into the portable polypropylene rotor vise (346133). Place the lid on the rotor and tighten it firmly to the right (clockwise) by hand. No tool is required.

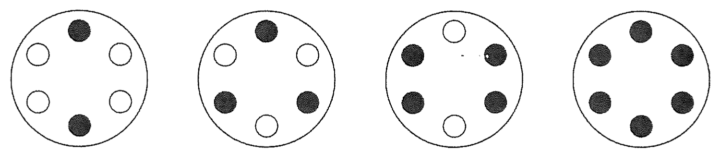
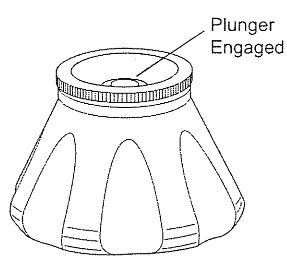


Figure 1. Arranging Tubes in the Rotor. Two, three, four, or six tubes can be centrifuged per run if they are arranged in the rotor as shown.

OPERATION



1. Use an absorbent towel to wipe off condensation from the rotor, then carefully place the rotor on the drive hub.

2. Lock the rotor in place by gently pressing the plunger down until you feel it click. When you remove your finger, the plunger will remain flush with the rotor body if it is properly engaged. If the plunger pops up, repeat the procedure. (The Optima MAX, MAX-XP, and MAX-E ultracentrifuges automatically secure the rotor to the drive shaft without the need for engaging the plunger.)

⚠ **CAUTION**

In all ultracentrifuge models except the Optima MAX, MAX-XP, and MAX-E, it is very important to lock the rotor in place before beginning the run to ensure that the rotor remains seated during centrifugation. Failure to lock the rotor in place before beginning the run may result in damage to both rotor and instrument.

3. Refer to the instrument instruction manual for ultracentrifuge operation.

4. For additional operating information, see the following:

   - RUN TIMES, page 10, for using *k* factors to adjust run durations.

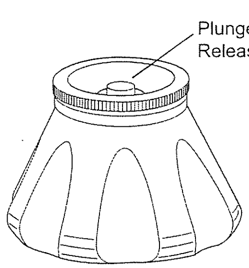
   - RUN SPEEDS, page 11, for information about speed limitations.

   - SELECTING CsCl GRADIENTS, page 13, 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. To release the plunger at the end of the run, gently press it down until you feel it click. When you remove your finger the plunger will pop up to its released position.

2. Remove the rotor from the ultracentrifuge and place it in the rotor vise.

3. Remove the lid by unscrewing it to the left (counterclockwise).

4. Use a tube removal tool to remove the spacers and tubes.

TUBES AND ACCESSORIES

The TLA-100.3 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.

Table 1. Available Tubes for the TLA-100.3 Rotor. Use only the items listed here and observe maximum fill volumes and speed shown.

Tube			Required Accessory		Max. Speed/ RCF/ *k* Factor
Dimensions and Volume	Description	Part Number	Description	Part Number	
13 × 32 mm 3.5 mL	Quick-Seal polyallomer	349621 (pkg/50)	Noryl[a] spacer	355937 (pkg/6)	100 000 rpm 541 000 × *g* 14
13 × 51 mm 3.0 mL	thickwall polycarbonate	349622 (pkg/25)	none	—	100 000 rpm 541 000 × *g* 16
13 × 51 mm 3.0 mL	thickwall polyallomer	349623 (pkg/25)	none	—	70 000 rpm[bc] 265 000 × *g* 34
13 × 25 mm 2.0 mL	Quick-Seal polyallomer	345829 (pkg/50)	Noryl spacer	360270 (pkg/6)	100 000 rpm 541 000 × *g* 11
11 × 38 mm 1.5 mL	Microfuge, polyallomer (capped)	357448 (pkg/500)	Delrin[d] adapter	355919 (pkg/8)	70 000 rpm[ce] 245 000 × *g* 24

[a] Noryl is a registered trademark of GE Plastics.

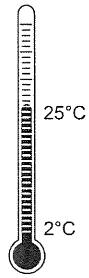
[b] Stress cracking and collapse can occur at high speeds. Discard these tubes after a single use.

[c] These maximum speeds were determined using water samples. Use of other solvents may require further speed reduction to prevent tube failure.

[d] Delrin is a registered trademark of E.I. Du Pont de Nemours & Company.

[e] At 40°C, speed must be reduced to 59 000 rpm.

Temperature Limits



- Plastic tubes have been centrifuge tested for use at temperatures between 2 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 2°C prior to centrifugation.

Quick-Seal® Tubes

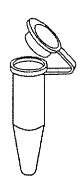
Quick-Seal tubes must be sealed prior to centrifugation. These tubes are heat sealed and do not need caps; however, spacers are required on top of the tubes when they are loaded into the rotor.

- Fill Quick-Seal tubes leaving a *small* bubble of air at the base of the neck. Do not leave a large air space—too much air can cause excessive tube deformation.
- Refer to *Rotors and Tubes* for detailed information on the use and care of Quick-Seal tubes.

Thickwall Tubes

Thickwall polyallomer and polycarbonate tubes can be run partially filled (at least half filled) without caps, but all opposing tubes for a run must be filled to the same level with liquid of the same density. Do not overfill capless tubes; be sure to note the reduction in run speed shown in Table 1.

Microfuge Tubes



The 1.5-mL microfuge tubes, with attached caps, are made of clear polyallomer. The tubes are placed in adapters for use in this rotor. All opposing tubes for a run must be filled with liquid of the same density. Be sure to note the run speed reduction shown in Table 1.

RUN TIMES

TIME HR:MIN

03:30

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:

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

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

After substitution:

$$k = \frac{(2.533 \times 10^{11})\ln(r_{max}/r_{min})}{\text{rpm}^2} \tag{2}$$

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).

$$t = \frac{k}{s} \tag{3}$$

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

$$k_{adj} = k\left(\frac{100\,000}{\text{actual run speed}}\right)^2 \tag{4}$$

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:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \tag{5}$$

For more information on k factors see *Use of* k *Factor for Estimating Run Times from Previously Established Run Conditions* (publication DS-719).

RUN SPEEDS

SPEED RPM/RCF

100 000 RPM

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.

Speeds must be reduced under the following circumstances:

1. If nonprecipitating solutions more dense than 1.7 g/mL are centrifuged, the maximum allowable run speed must be reduced according to the following equation:

$$\text{reduced maximum speed} = (100\ 000\ \text{rpm}) \sqrt{\frac{1.7\ \text{g/mL}}{\rho}} \qquad (6)$$

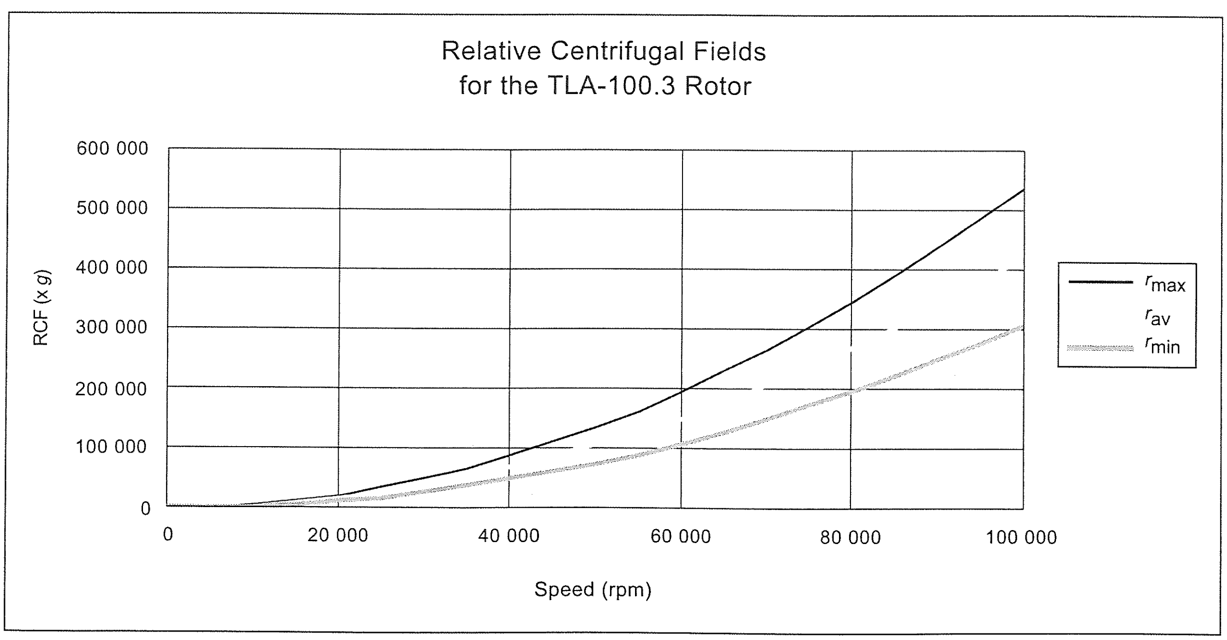
   where ρ is the density of the tube contents. This speed reduction will protect the rotor from excessive stresses due to the added tube load. *Note, however, that the use of this formula may still produce maximum speed values that are higher than the limitations imposed by the use of certain tubes or adapters.* In such cases, use the lower of the two values.

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. Precipitation during centrifugation would alter the density distribution of CsCl and this would change the position of the sample bands. Figures 2 and 3, together with the description and examples below, show how to reduce run speeds when using CsCl gradients.

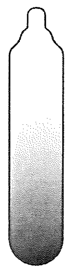
Table 2. Relative Centrifugal Fields (3.5-mL Quick Seal Tubes) for the TLA-100.3 Rotor. Entries in this table are calculated from the formula $RCF = 1.12r\,(RPM/1000)^2$ and then rounded to three significant digits.

Rotor Speed (rpm)	Relative Centrifugal Field (× *g*)			*k* Factor*
	At r_{max} (48.3 mm)	At r_{av} (37.9 mm)	At r_{min} (27.5 mm)	
100 000	541 000	424 000	308 000	14
95 000	488 000	383 000	278 000	16
90 000	438 000	344 000	249 000	18
85 000	391 000	307 000	223 000	20
80 000	346 000	272 000	197 000	22
75 000	304 000	239 000	173 000	25
70 000	265 000	208 000	151 000	29
65 000	229 000	179 000	130 000	34
60 000	195 000	153 000	111 000	39
55 000	164 000	128 000	93 000	47
50 000	135 000	106 000	77 000	57
45 000	110 000	86 000	63 000	70
40 000	87 000	68 000	49 300	89
35 000	66 300	52 000	38 000	116
30 000	49 000	38 200	28 000	158
25 000	33 800	26 500	19 300	227
20 000	21 600	17 000	12 300	355

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



SELECTING CsCl GRADIENTS



Precipitation during centrifugation would alter density distribution, and this would change the position of the sample bands. Curves in Figures 2 and 3 are provided up to the maximum rated speed of the rotor.

NOTE

The curves in Figures 2 and 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.

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 2a or 3a ensure that CsCl will not precipitate during centrifugation in the TLA-100.3 rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves).

The reference curves in Figures 2b and 3b show gradient distribution at equilibrium. Each curve in Figure 2b or 3b is within the density limits allowed for the TLA-100.3 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 2b or 3b 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 2a or 3a.) Figure 2b or 3b can also be used to approximate the banding positions of sample particles.

ADJUSTING FILL VOLUMES

Figures 2 and 3 show that several fill volumes are possible in a tube. If a 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. 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.

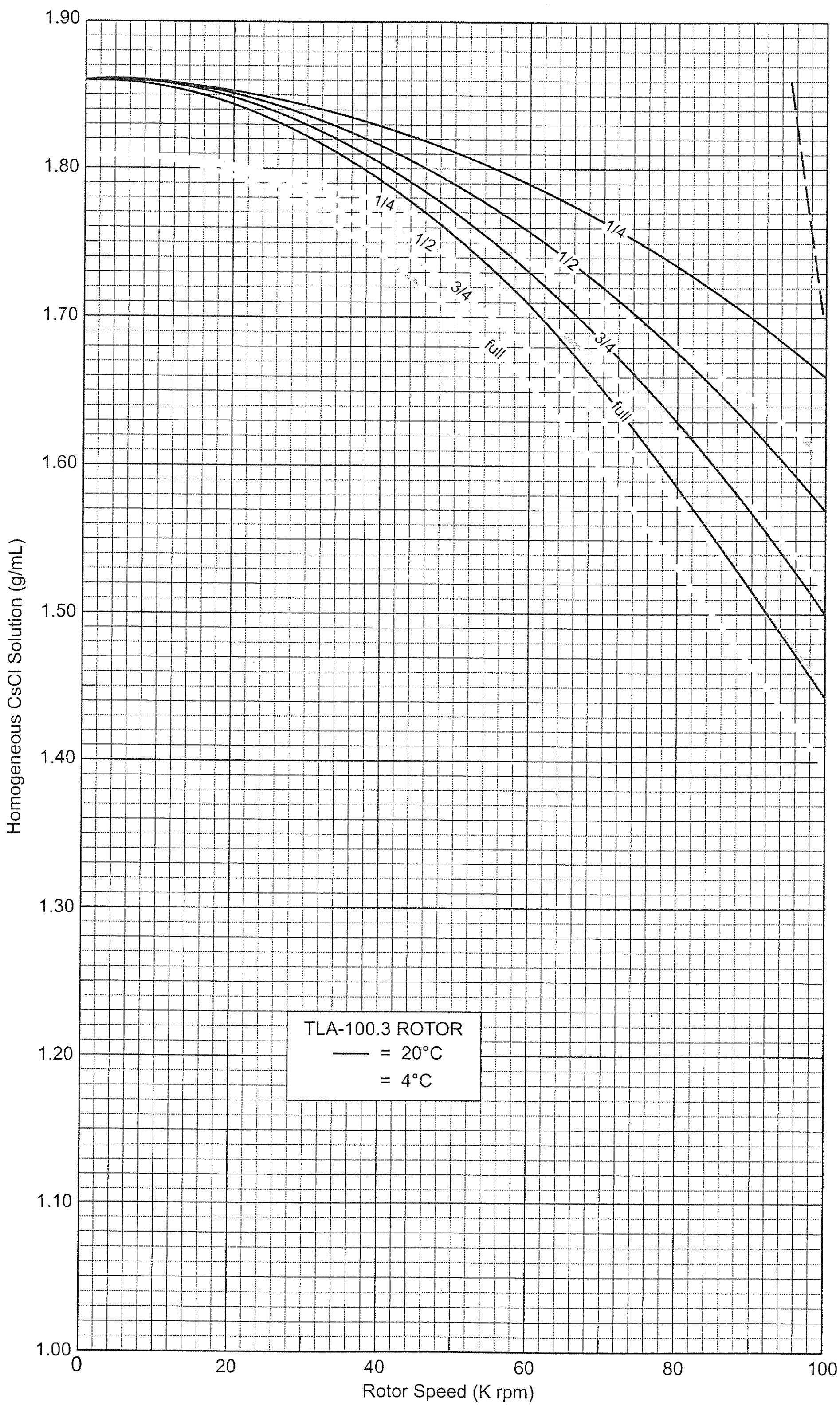


Figure 2a. Precipitation Curves (3.5-mL Quick-Seal Tubes) for the TLA-100.3 Rotor. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation. The dashed line is a representation of equation (6), and is shown here to illustrate the inability of that equation to guard against CsCl precipitation.

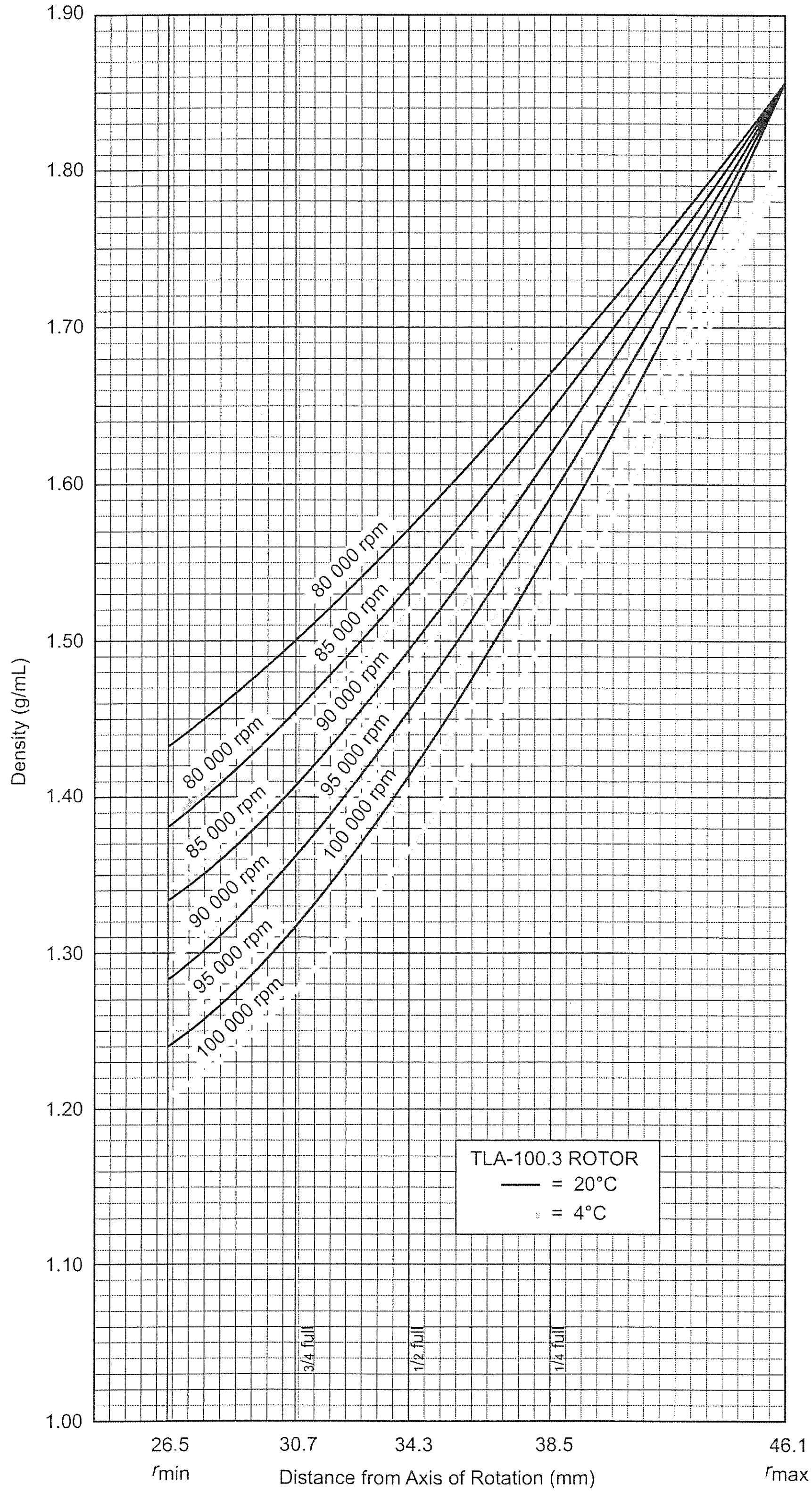


Figure 2b. CsCl Gradients at Equilibrium (3.5-mL Quick-Seal Tubes). Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 2a) results in gradients presented here.

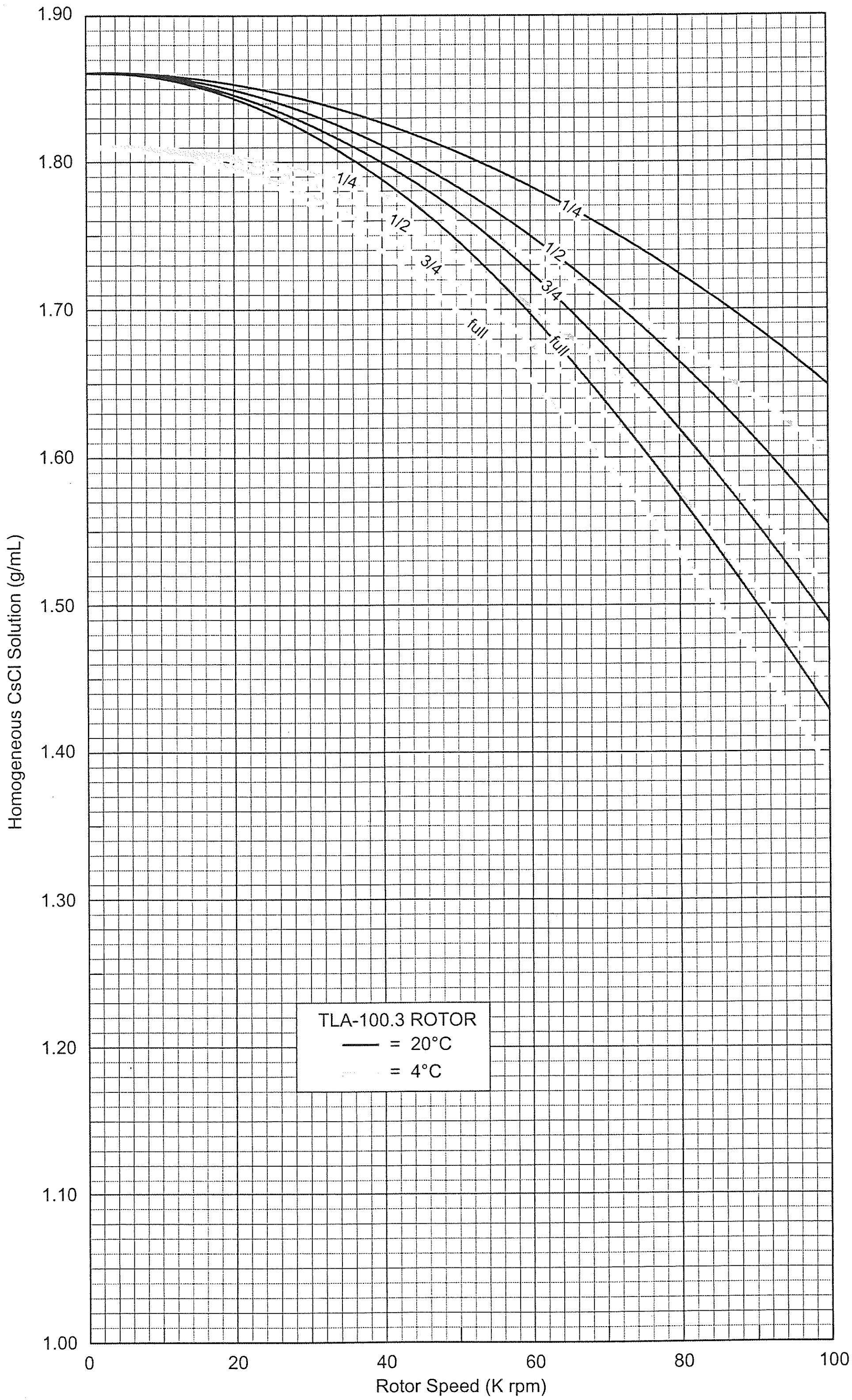


Figure 3a. Precipitation Curves (Open Top Tubes) for the TLA-100.3 Rotor. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation.

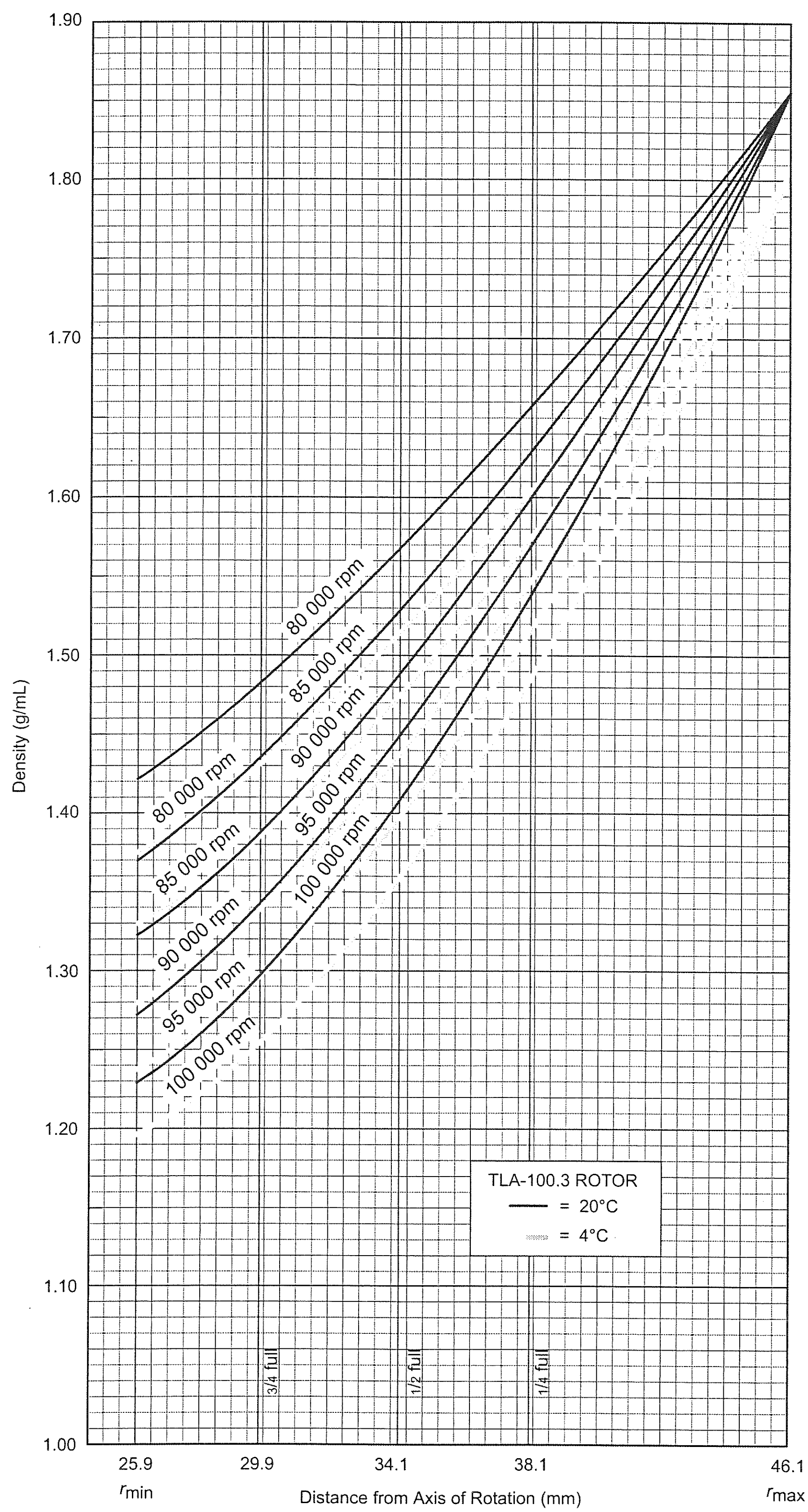
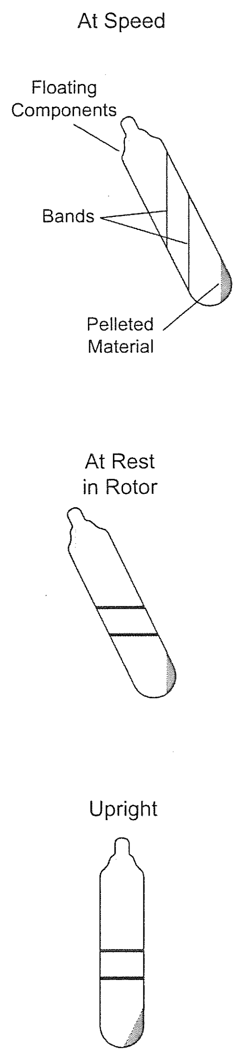


Figure 3b. CsCl Gradients at Equilibrium (Open Top Tubes). Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 3a) results in gradients presented here.

For example, a *three-quarter-filled* tube of 1.55-g/mL homogeneous CsCl solution at 20°C may be centrifuged at 90 000 rpm (see Figure 3a). The segment of the 90 000-rpm curve (Figure 3b) from the three-quarter-filled line to 1.86-g/mL at the tube bottom represents this gradient. The same solution in a *full* tube may be centrifuged no faster than 83 000 rpm.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS



Example A: Knowing homogeneous CsCl density (1.58 mL) and approximate particle buoyant densities (1.58 and 1.60 g/mL), where will particles band (using Quick-Seal tubes)?

1. In Figure 2a, find the curve that corresponds to the required run temperature (20°C) and fill volume (full). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (80 000 rpm).

2. In Figure 2b, 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.

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

In this example, particles will band about 11.0 and 9.8 mm from the tube bottom, about 1.2 mm of centerband-to-centerband separation at the rotor's 30-degree tube angle. When the tube is held upright, there will be about 1.38 mm of centerband-to-centerband separation. This interband distance, d_{up}, can be calculated from the formula:

$$d_{up} = \frac{d_\theta}{\cos\theta} \qquad (7)$$

where d_θ is the interband distance when the tube is held at an angle, θ, in the rotor.

Example B: Knowing particle buoyant densities (e.g., 1.55 and 1.57 g/mL), how do you achieve good separation (using open top tubes)?

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

2. Select the curve at the required temperature (20°C) and fill volume (full) that gives the best particle separation.

3. Note the run speed along the selected curve (80 000 rpm).

4. From Figure 3a, select the maximum homogeneous CsCl density that corresponds to the temperature and run speed established above. These parameters will provide the particle-banding pattern selected in Step 2.

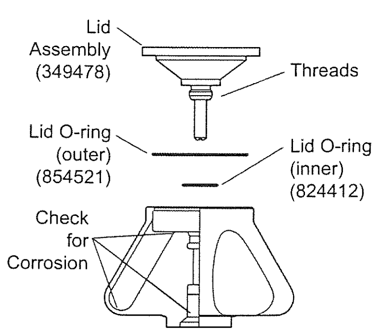
In this example, particles will band at about 12.8 and 11.8 mm from the bottom of the tube (about 1 mm apart). When the tube is held upright there will be about 1.15 mm of center-of-band to center-of-band separation.

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.

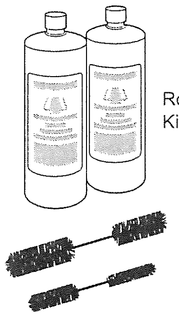


- Regularly lubricate the metal threads in the rotor with a thin, even coat of Spinkote lubricant. Failure to keep these threads lubricated can result in damaged threads.

- Regularly apply silicone vacuum grease to the O-rings. Replace O-rings about twice a year or whenever worn or damaged.

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.

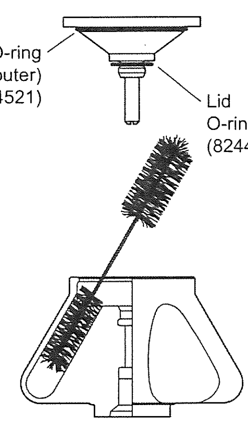
Under normal use, wash the rotor frequently (at least weekly) to prevent buildup of residues.

1. Remove the O-rings before washing.

2. Wash the rotor and lid in a mild detergent, such as Beckman Solution 555™ (339555), that won't damage the rotor. The Rotor Cleaning Kit contains two plastic-coated brushes and two quarts of Solution 555 for use with rotors and accessories. Dilute the detergent 10 to 1 with water.

NOTE

Do not wash rotor components in a dishwasher. Do not soak in detergent solution for long periods, such as overnight.



3. Rinse the cleaned rotor and components with distilled water.

4. Air-dry the rotor and lid upside down. *Do not use acetone to dry the rotor.*

5. Apply a thin, even coat of silicone vacuum grease to both lid O-rings before replacing them in the grooves in the lid.

Clean metal threads as necessary (at least every 6 months). Use a brush and concentrated Solution 555. Rinse and dry thoroughly, then lubricate lightly but evenly with Spinkote to coat all threads.

Periodically remove the O-rings and wipe clean as necessary. Clean the O-ring grooves with a cotton-tipped swab. Reapply a light film of silicone vacuum grease.

SUPPLY LIST

NOTE

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

Contact Beckman Coulter Sales (1-800-742-2345 in the United States; worldwide offices are listed on the back cover of this manual) or see the Beckman Coulter *Ultracentrifuge Rotors, Tubes & Accessories* catalog (BR-8101) for detailed information on ordering parts and supplies. For your convenience, a partial list is given below.

REPLACEMENT ROTOR PARTS

TLA-100.3 rotor assembly	349481
Lid assembly	349478
Lid O-ring (outer)	854521
Lid O-ring (inner)	824412
Cap and plunger assembly	349477
Spring	347903
Rotor vise	346133

OTHER

Tubes and accessories	see Table 1
Tube rack	355872
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	348122
Spacer removal tool (for 3.5-mL and 2.0-mL Quick-Seal tubes)	338765
Tube removal tool	361668
Curved hemostat (6-in.)	927208
Straight hemostat (6-in.)	961519
Fraction Recovery System	342025
Fraction Recovery System Adapter Kit for TL-series tubes	347828
Beckman Coulter CentriTube Slicer	347960
CentriTube Slicer replacement blades (pkg of 10)	348299

CentriTube Slicer adapter (for 13-mm tubes)	354526
Spinkote lubricant (2 oz)	306812
Silicone vacuum grease (1 oz)	335148
Rotor Cleaning Kit	339558
Rotor cleaning brush	347404
Beckman Solution 555 (1 qt)	339555