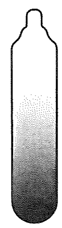
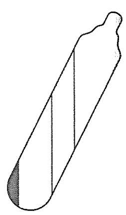
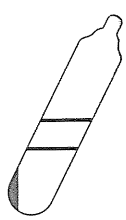
BECKMAN COULTER®

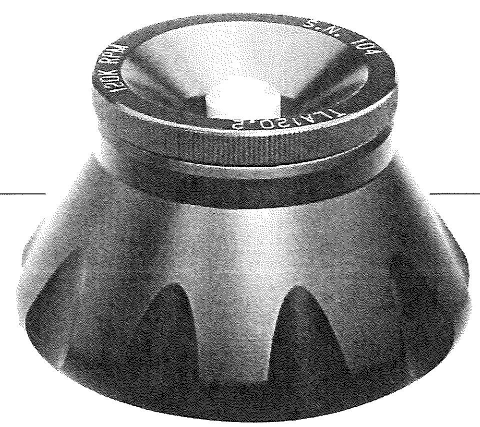
TL-TB-016AH
August 2009

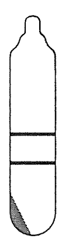




TLA-120.2 Rotor

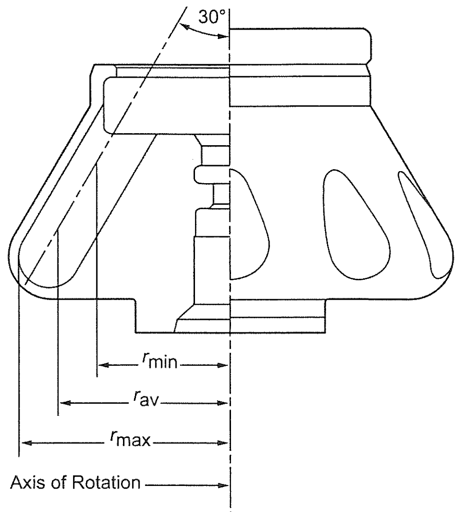






Used In Beckman Coulter Optima™ MAX-XP, MAX, MAX-E, TL, TLX, and TL-100 Tabletop Ultracentrifuges

TLA-120.2 ROTOR



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

SPECIFICATIONS

Maximum speed	120 000 rpm
Density rating at maximum speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (38.9 mm)	627 000 × *g*
At r_{av} (31.8 mm)	513 000 × *g*
At r_{min} (24.5 mm)	395 000 × *g*
k factor at maximum speed	8
Conditions requiring speed reductions	see RUN SPEEDS
Number of tube cavities	10
Available tubes	see Table 1
Nominal tube dimensions (largest tube)	11 × 34 mm
Nominal tube capacity (open-top)	1.0 mL
Nominal tube capacity (Quick-Seal®)	2.0 mL
Nominal rotor capacity	20 mL
Approximate acceleration time to maximum speed (fully loaded)	2 1/2 min
Approximate deceleration time from maximum speed (fully loaded)	2 min
Weight of fully loaded rotor	0.6 kg (1.2 lb)
Rotor material	titanium

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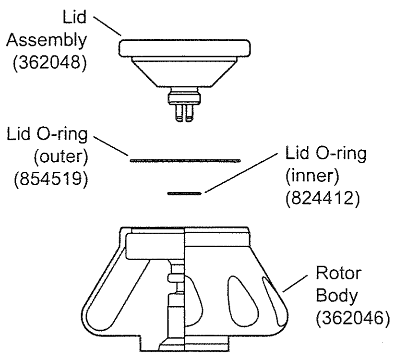
* Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed ($r\omega^2$) to the standard acceleration of gravity (*g*) according to the following formula:

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

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 (9807 mm/s²). After substitution:

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

DESCRIPTION



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

The TLA-120.2 fixed angle rotor, rated for 120 000 rpm, has a tube angle of 30 degrees from the axis of rotation. The rotor can centrifuge up to 10 tubes and is used in Beckman Coulter Optima™ MAX, MAX-E, MAX-XP, 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.

This rotor was tested* to demonstrate containment of microbiological aerosols under normal operating conditions of the associated Beckman Coulter centrifuge, when used and maintained as instructed.

The rotor is specially designed with a fluid-containment annulus located below the O-ring sealing surface (see Figure 1). The annulus retains fluid that may escape from leaking or overfilled tubes, thereby preventing the liquid from escaping into the instrument chamber.

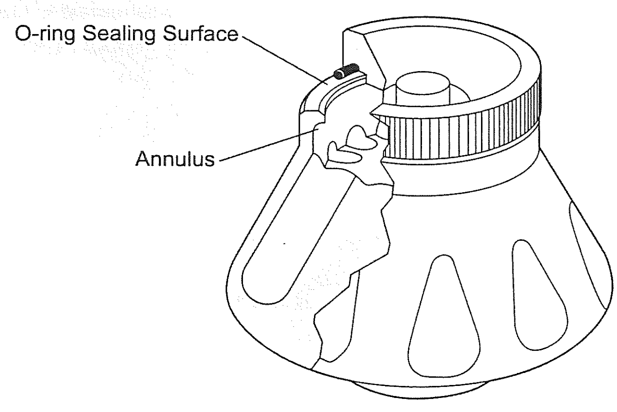


Figure 1. Fluid-Containment Annulus

* Validation of microbiological containment was done at an independent third-party testing facility (CAMR, Porton Down, UK, or USAMRIID, Ft. Detrick MD, U.S.A.). Improper use or maintenance may affect seal integrity and thus containment.

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-120.2 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-120.2 rotor, their safety in this rotor cannot be ascertained by Beckman Coulter. Use of other manufacturers' components or accessories in the TLA-120.2 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

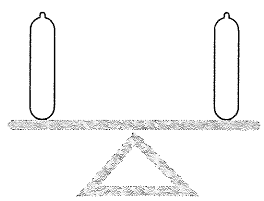
Read the Safety Notice page at the front of this manual before using the rotor.

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. Load the filled and sealed (if required) tubes symmetrically into the rotor (see page 8 for tube information). If fewer than ten tubes are being run, they must be arranged symmetrically in the rotor (see Figure 2). *Opposing tubes must be filled to the same level with liquid of the same density.*
4. Use the required spacers, if necessary (see Table 1), to complete the loading operation.
5. 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.

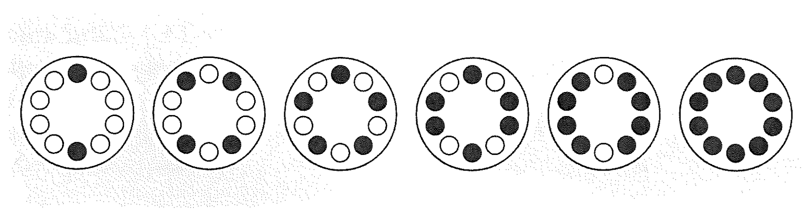
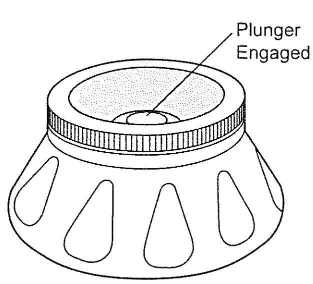


Figure 2. Arranging Tubes in the Rotor. Two, four, five, six, eight, or ten tubes can be centrifuged per run if they are arranged in the rotor as shown.

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 secures 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 9, for using *k* factors to adjust run durations.

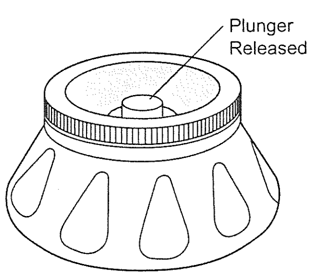
   - RUN SPEEDS, page 10, for information about speed limitations.

   - SELECTING CsCl GRADIENTS, page 12, 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

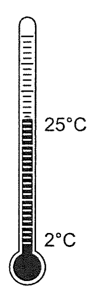
The TLA-120.2 rotor uses tubes and accessories listed in Table 1. Be sure to use only those items listed, and to observe the maximum speed limits shown. Refer to Appendix A in *Rotors and Tubes* for information on the chemical resistances of tube and accessory materials.

Table 1. Available Tubes for the TLA-120.2 Rotor.
Use only the items listed here and observe maximum fill volumes and speeds shown.

Tube			Required Accessory		Max. Speed/ RCF/ *k* Factor
Dimensions and Volume	Description	Part Number	Description	Part Number	
11 × 34 mm 1.0 mL	thickwall polycarbonate	343778 (pkg/100)	none	—	120 000 rpm 627 000 × *g* 8
11 × 34 mm 1.0 mL	thickwall polyallomer	347287 (pkg/100)	none	—	80 000 rpm 279 000 × *g* 18
11 × 25 mm 1.5 mL	Quick-Seal polyallomer	344624 (pkg/50)	Noryl* spacer	344636 (pkg/6)	120 000 rpm 627 000 × *g* 14
11 × 32 mm 2.0 mL	Quick-Seal polyallomer	344625 (pkg/50)	Noryl spacer	344674 (pkg/6)	120 000 rpm 627 000 × *g* 16

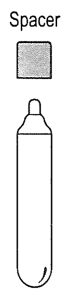
* Noryl is a registered trademark of GE Plastics.

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



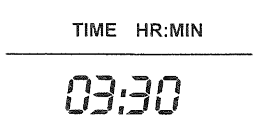
Quick-Seal tubes must be sealed prior to centrifugation. These tubes are heat sealed and do not need caps; however, spacers are required on top of the tubes when they are loaded into the rotor.

- 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.

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:

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

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:

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

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).

$$t = \frac{k}{s} \qquad (3)$$

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

$$k_{adj} = k \left(\frac{120\ 000}{\text{actual run speed}} \right)^2 \qquad (4)$$

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:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \qquad (5)$$

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

120 000 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.

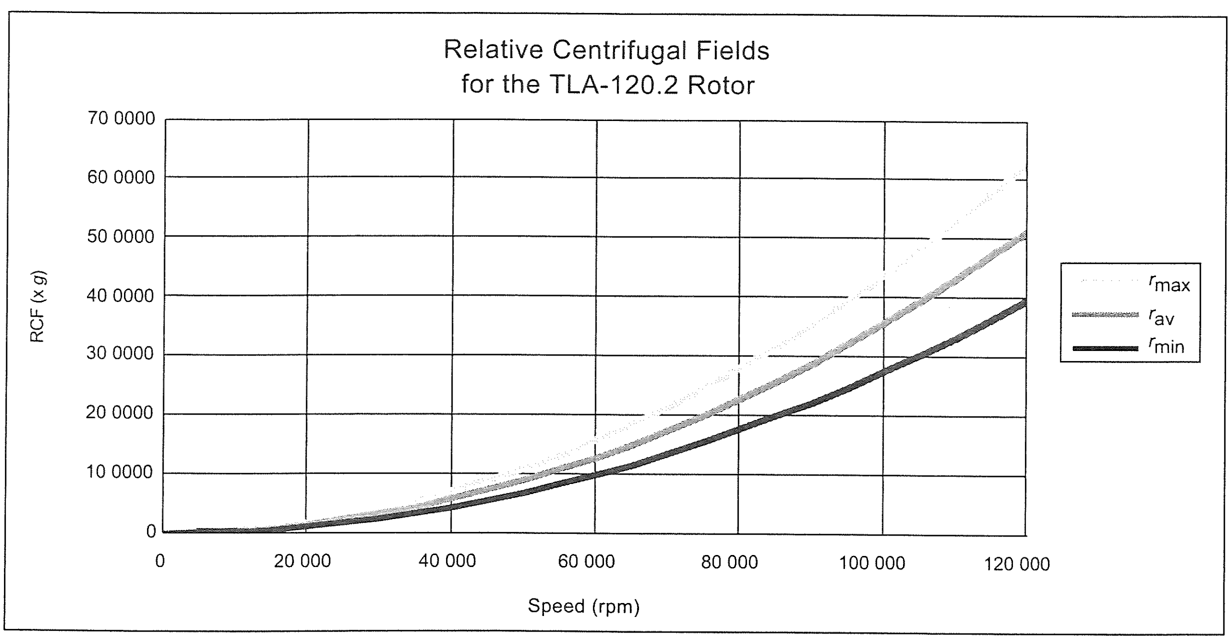
Table 2. Relative Centrifugal Fields for the TLA-120.2 Rotor. Entries in this table are calculated from the formula

$$RCF = 1.12r\ (RPM/1000)^2$$

and then rounded to three significant digits.

Rotor Speed (rpm)	Relative Centrifugal Field (× *g*)			*k* Factor*
	At r_{max} (38.9 mm)	At r_{av} (31.8 mm)	At r_{min} (24.5 mm)	
120 000	627 000	513 000	395 000	8
115 000	576 000	471 000	363 000	9
110 000	527 000	431 000	332 000	10
105 000	480 000	393 000	303 000	11
100 000	436 000	356 000	274 000	12
95 000	393 000	321 000	248 000	13
90 000	353 000	288 000	222 000	14
85 000	315 000	257 000	198 000	16
80 000	279 000	228 000	176 000	18
75 000	245 000	200 000	154 000	21
70 000	213 000	175 000	134 000	24
65 000	184 000	150 000	116 000	28
60 000	157 000	128 000	98 800	33
55 000	132 000	108 000	83 000	39
50 000	109 000	89 000	68 600	47

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



Speeds must be reduced under the following circumstances:

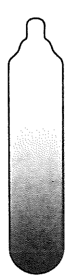
1. If nonprecipitating solutions more dense than 1.7 g/mL are centrifuged, the maximum allowable run speed must be reduced according to the following equation:

$$\text{reduced maximum speed} = (120\,000 \text{ rpm})\sqrt{\frac{1.7 \text{ g/mL}}{\rho}} \qquad (6)$$

   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 3 and 4, together with the description and examples below, show how to reduce run speeds when using CsCl gradients.

SELECTING CsCl GRADIENTS



Precipitation during centrifugation would alter density distribution, and this would change the position of the sample bands. Curves in Figures 3 and 4 are provided up to the maximum rated speed of the rotor. (Curves in Figures 3 and 4 were generated using the 1.5-mL Quick-Seal tube.)

NOTE

The curves in Figures 3 and 4 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 3 ensure that CsCl will not precipitate during centrifugation in the TLA-120.2 rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves).

The reference curves in Figure 4 show gradient distribution at equilibrium. Each curve in Figure 4 is within the density limits allowed for the TLA-120.2 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 4 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 4 can also be used to approximate the banding positions of sample particles.

ADJUSTING FILL VOLUMES

Figures 3 and 4 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.

For example, a *one-quarter-filled* tube of a 1.70 g/mL homogeneous CsCl solution at 20°C may be centrifuged at 105 000 rpm (see Figure 4). The segment of the 105 000 rpm curve (Figure 5) from the one-quarter-filled line to 1.86 g/mL at the tube bottom represents this gradient. The same solution in a *three-quarter-filled* tube may be centrifuged at 84 000 rpm; Figure 5 presents the gradient profile (use the three-quarter-filled segment only). A tube *full* of the 1.70 g/mL CsCl solution may be centrifuged no faster than 79 000 rpm.

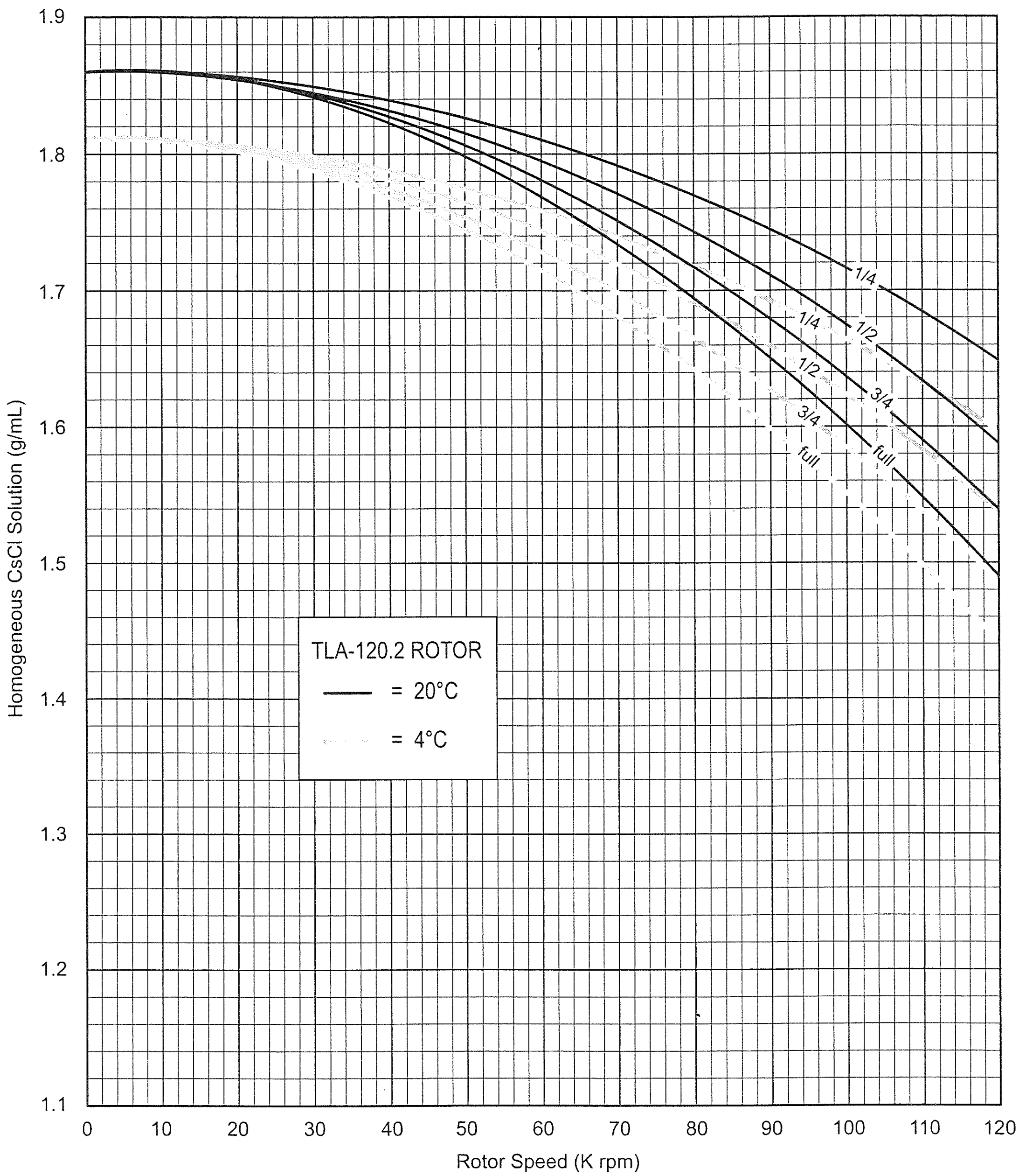


Figure 3. Precipitation Curves for the TLA-120.2 Rotor.
Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation.

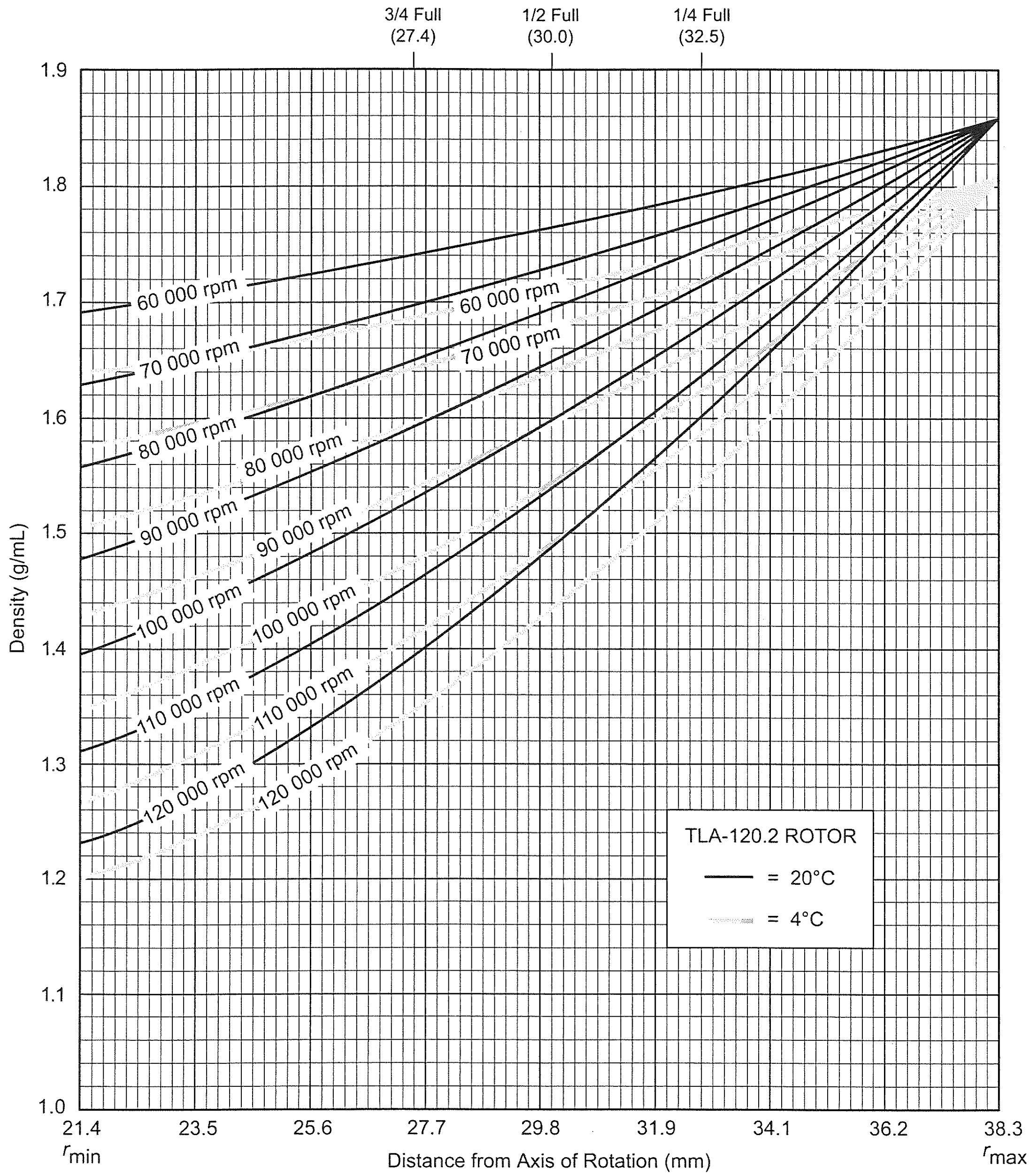
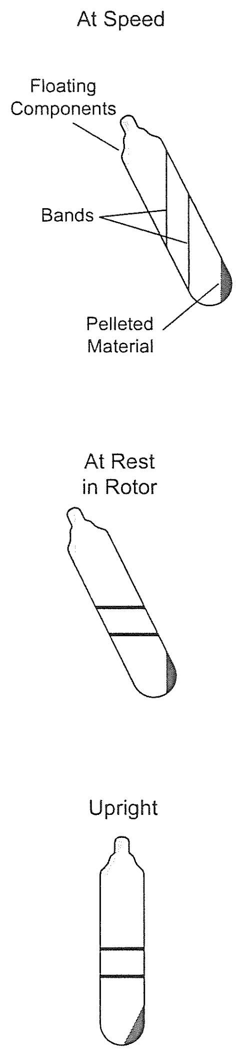


Figure 4. CsCl Gradients at Equilibrium.
Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 3) results in gradients presented here.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS



Example A: A separation that is done frequently is the banding of plasmid DNA in cesium chloride with ethidium bromide. The starting density of the CsCl solution is 1.55 g/mL. In this separation the covalently closed, circular plasmid bands at a density of 1.57 g/mL, while the nicked and linear species band at 1.53 g/mL. At 20°C, where will particles band?

1. In Figure 3, 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 (110 000 rpm).

2. In Figure 4, 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 32.1 and 30.0 mm from the tube bottom, about 2.1 mm of centerband-to-centerband separation at the rotor's 30-degree tube angle. When the tube is removed from the rotor and held upright (vertical and stationary), there will be about 2.4 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 (for example, 1.66 and 1.62 g/mL), how do you achieve good separation (using open top tubes)?

1. In Figure 4, sketch in a horizontal line corresponding to each particle's buoyant density.

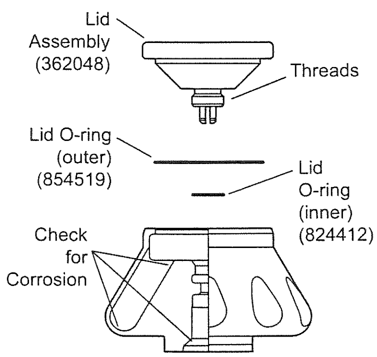
2. Select the curve at the required temperature (20°C) and fill volume that gives the best particle separation.

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

4. From Figure 4, 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.

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.

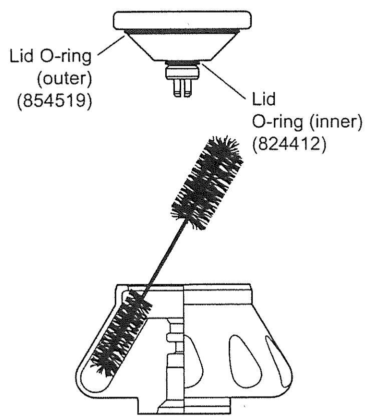
Under normal use, wash the rotor frequently (at least weekly) to prevent buildup of residues.

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.

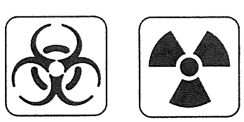
4. Air-dry the rotor and lid upside down. *Do not use acetone to dry the rotor.*

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.

DECONTAMINATION



If the rotor or other components are contaminated with radioactive, toxic, or pathogenic materials, follow appropriate decontamination procedures as outlined by appropriate laboratory safety guidelines and/or other regulations. Consult Appendix A in *Rotors and Tubes* to select an agent that will not damage the rotor.

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, available at www.beckmancoulter.com) for detailed information on ordering parts and supplies. For your convenience, a partial list is given below.

REPLACEMENT ROTOR PARTS

TLA-120.2 rotor assembly 362046
Lid assembly 362048
Lid O-ring (outer) 854519
Lid O-ring (inner) 824412
Rotor vise 346133

OTHER

Tubes and accessories . . . . see Table 1
Tube rack. 349387
Quick-Seal Cordless Tube Topper kit, 60 Hz 358312
Quick-Seal Cordless Tube Topper kit, 50 Hz (Europe) 358313
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Curved hemostat (6-in.). 927208
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Beckman Coulter CentriTube Slicer 347960
CentriTube Slicer replacement blades (pkg of 10) 348299
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