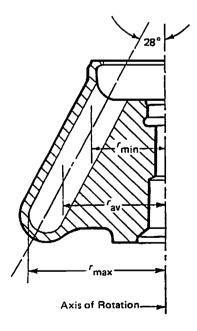
INSTRUCTIONS FOR USING THE TLA-100.4 FIXED ANGLE ROTOR

In the Beckman Optima™ Series TL and TLX and Modified* TL-100
Tabletop Ultracentrifuges





SPECIFICATIONS

Maximum speed	m
Density rating at full speed 1.7 g/m	L
Relative Centrifugal Field† at maximum speed	
(3.2-mL polycarbonate tubes):	
At r_{max} (48.5 mm)	g
At r _{av} (37.2 mm)	g
At r _{min} (26.0 mm)	g
k factor at maximum speed (3.2-mL polycarbonate tubes) 1	
Number of tube cavities	8
Available tubes see Table	1
Nominal dimensions of largest tube 13 x 51 mm (½ x 2 in	ı.)
Nominal tube capacity of largest tube 5.1 m	L
Nominal rotor capacity 40.8 m	L
Approximate acceleration time to maximum speed 3 ½ mi	in
Approximate deceleration time from maximum speed 2 1/2 mi	in
Weight of fully loaded rotor 1.02 kg (2.25 lt	b)
Rotor material titanium	
Conditions requiring speed reductions see RUN SPEED	S

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

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

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

^{*} See the CAUTION under DESCRIPTION.

[†] 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:

DESCRIPTION

The TLA-100.4, rated for 100 000 rpm, is a fixed angle rotor with a tube angle of 28 degrees from the axis of rotation. The rotor can centrifuge up to eight tubes and is used in Beckman Optima TM Series TL and TLX tabletop ultracentrifuges. This rotor can also be used in TL-100 tabletop ultracentrifuges that have modification kit 360477 installed (contact your Beckman Field Service representative to have the modification kit installed).

CAUTION Before the TLA-100.4 rotor is used in a TL-100 ultracentrifuge, the instrument must be updated with a new drive spindle and updated operating software (modification kit number 360477). Operation of the TLA-100.4 rotor in an unmodified TL-100 may cause the rotor to stick or slip on the spindle.

The TLA-100.4 rotor develops centrifugal forces that are suitable for rapid pelleting and isopycnic separations. Up to 40.8 mL of gradient and sample can be centrifuged per run.

The rotor is made of titanium and is finished with black polyurethane paint. The lid is aluminum and is black-anodized for corrosion resistance. A plunger in the lid locks the rotor to the drive hub before the run begins. Two lubricated O-rings made of Buna N maintain atmospheric pressure inside the rotor during centrifugation.

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.

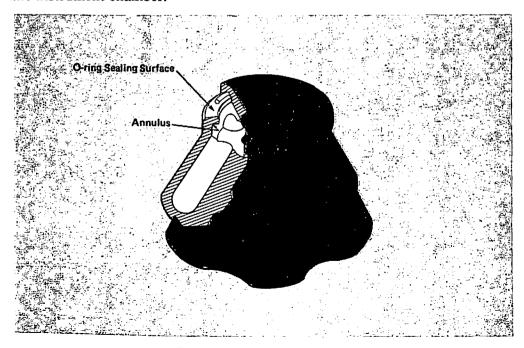


Figure 1. Fluid-Containment Annulus

The centrifuge identifies refor speed during the run by means of a magnetic speed sensor system in the rotor chamber of the instrument and magnets on the bottom of the rotor. This overspeed protection system ensures that the rotor does not exceed its permitted speed. The TLA-100.4 rotor is warranted for 5 years (see the Warranty).

PREPARATION AND USE

& Company

NOTE Specific information about the TLA-100.4 fixed angle rotor is given here. Use the appropriate instrument instruction manual together with this bulletin for complete rotor and accessory operation.

TUBES

Tubes that may be used in the TLA-100.4 rotor are listed in Table 1. Be sure to observe the maximum rotor speed limits and fill volumes shown. The g-MaxTM system 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 Quick-Seal tubes listed in Table 1 in the TLA-100.4 rotor without reduction in g force. For detailed information on the g-Max system see publication DS-709. Refer to publication IN-175, Chemical Resistances, for information on the chemical resistances of tube and accessory materials.

Table 1. Available Tubes for the TLA-100.4 Rotor. Use only the items listed here.

Description	Pärt Number	Dimensions (in./mm)	Fill Vol. (mL)		Required Accessory		Maximum Speed (rpm)/
			Maximum	Minimum	Part Number	Description	RCF/=- k Factor
Quick-Seal polyallomer	362248 pkg/50	½ x 2 (13 x 51)	5.1	5.1	362307 set/8	floating spacer*	100 000 543 000 x g 25
thickwall polycarbonate	362305 pkg/50	½ x 2 ¼ (13 x 56)	3.2	1.6	none	none	100 000 543 000 x g 16
thickwail polyalicmer	362333 pkg/50	½ x 2 ¼ (13 x 56)	3.2	3.2	none	none	70 000 266 000 x g 32
Quick-Seal polyallomer	349621 pkg/50	½ x 1 ¼ (13 x 32)	3.5	3.5	360270 set/8	floating spacer [†]	100 000 543 000 x g 16
Quick-Seal polyallomer	345829 pkg/50	½ x 1 (13 x 25)	2.0	2.0	360270 set/8	floating spacer i	100 000 543 000 x g 11

^{*}These floating spacers are made of Delrin, a registered trademark of E.I. Du Pont de Nemours & Company.

[†]These floating spacers are made of Noryl, a registered trademark of General Electric.

Temperature Limits

Polyallomer and polycarbonate tubes have been centrifuge tested for use at temperatures between 2 and 25°C. For centrifugation at other temperatures, they should be pretested under anticipated run conditions. Do not freeze polyallomer tubes before centrifugation as they may become brittle and crack.

Filling and Sealing Tubes

Thickwall polycarbonate and polyallomer tubes do not need caps. These tubes fit directly into the rotor without adapters or spacers. Be sure to observe the fill volumes and maximum speeds for these tubes.

Quick-Seal tubes have the greatest fill capacity and are hermetically heat sealed prior to centrifugation. Quick-Seal tubes should be filled 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 or collapse. If a Quick-Seal tube is only partially filled, a low-density immiscible liquid such as mineral oil must be floated on the tube contents to fill the tube to its maximum volume. Spacers must be placed on top of the tubes when they are loaded into the rotor. Refer to publication IN-163 or IN-181 for detailed information on the use and care of Quick-Seal tubes.

ROTOR PREPARATION

- Be sure that metal threads in the rotor are lightly but evenly lubricated with Spinkote™ lubricant, and the two O-rings are lightly but evenly coated with silicone vacuum grease.
- For runs at other than room temperature, always refrigerate or warm the rotor beforehand for fast equilibration.

Load the filled and sealed tubes symmetrically into the rotor, using the required spacers if necessary (see Table 1). If fewer than eight tubes are being run, they must be positioned symmetrically around the center of the rotor. Fill opposing tubes to the same level with liquid of the same density.

After the rotor is loaded, place the lid on the rotor and tighten it firmly (clockwise) by hand. No tool is required.

ROTOR INSTALLATION

Remove external moisture from the rotor by blotting with an absorbent towel just prior to installation. Carefully place the rotor on the drive hub and lock it in place by gently pressing the plunger in the rotor lid down until you hear a click. When you remove your finger, the plunger will remain flush with the lid (see Figure 2) if it is properly engaged. If the plunger pops up, repeat the procedure. It is very important to lock the rotor in place before beginning the run. Consult the instrument instruction manual for ultracentrifuge operation.

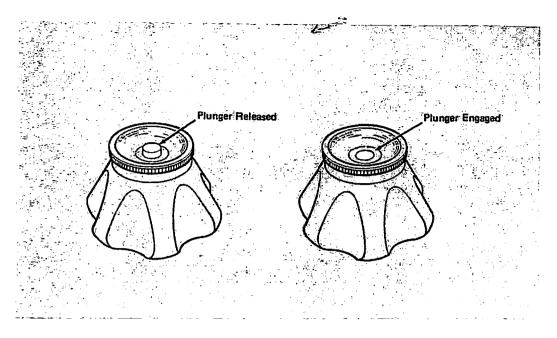


Figure 2. The Plunger in Locked and Released Positions in the Rotor Lid

REMOVAL AND SAMPLE RECOVERY

To release the plunger at the end of the run, gently press it down into the lid until you hear a click. When you remove your finger the plunger will pop up to its released position (see Figure 2). Remove the rotor from the instrument and remove the rotor lid by unscrewing it (counterclockwise) by hand. Use a hemostat or forceps to remove the tubes.

An adapter kit for TL-series tubes is available for the 342025 and 343890 Beckman Fraction Recovery Systems. The Beckman CentriTube Slicer is also designed to recover sample fractions from small plastic tubes. (See the Supply List for part numbers.) Contact your Beckman Representative to obtain information about these systems.

RUN TIMES

The k factor of the rotor is a measure of the rotor's pelleting efficiency. (Beckman 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:

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

where ω is the angular velocity of the rotor in radians per second ($\omega = 0.105 \text{ x}$ 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).

$$t = \frac{k}{s} \tag{2}$$

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

$$k_{\text{adj}} = k \left(\frac{100\ 000}{\text{actual run speed}} \right)^2 \tag{3}$$

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, a and b:

$$\frac{t_{\mathbf{a}}}{t_{\mathbf{b}}} = \frac{k_{\mathbf{a}}}{k_{\mathbf{b}}} \tag{4}$$

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

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 may then be described as having been subjected to the same force. The RCF at a number of rotor speeds (using 3.2-mL polycarbonate tubes) is provided in Table 2 and graphically shown in Figure 3. (Do not select rotational speeds that exceed the limits provided in Table 1.)

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

Roter	Relative			
Speed (rpm)	At r _{max} (48.5 mm)	At r _{av} (37.2 mm)	At r _{min} (26.0 mm)	Factor
100 000	543 000	417 000	291 000	16
95 000	490 000	376 000	263 000	17
90 000	440 000	337 000	236 000	19
85 000	392 000	301 000	210 000	22
80 000	348 000	267 000	186 000	25
75 000	306 000	234 000	164 000	28
70 000	266 000	204 000	143 000	32
65 000	230 000	176 000	123 000	37
60 000	196 000	150 000	105 000	44
55 000	164 000	126 000	88 100	52
50 000	136 000	104 000	72 800	63
45 000	110 000	84 400	59 000	78
40 000	86 900	66 700	46 600	99

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

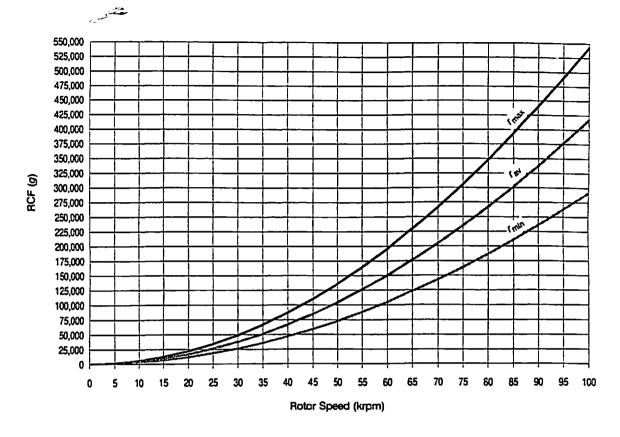


Figure 3. Relative Centrifugal Fields Using 3.2-mL Polycarbonate Tubes

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

reduced maximum speed =
$$(100\ 000\ \text{rpm})\sqrt{\frac{1.7\ \text{g/mL}}{\text{density of tube contents}}}$$
 (5)

Further speed limits must be imposed when CsCl or other self-forming-gradient salts are centrifuged, as equation (5) 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 4 and 5, together with the description and examples below, show how to reduce run speeds when using CsCl gradients.

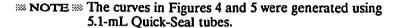
SELECTING CsCI GRADIENTS

NOTE::: The curves in Figures 4 and 5 are for solutions of CsCl salt only. If other salts are present in significant concentrations, the overall CsCl concentration may need to be reduced.

Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation may cause rotor failure. Precipitation will also alter density distribution, and therefore

sample separation. In general, lower speeds provide better resolution, but longer run times will be required to achieve particle separation and gradient equilibrium. Curves are provided up to the maximum rated speed of the rotor, but note also that tubes must never be centrifuged faster than the limits in Table 1.

Rotor speed is used to control the slope of a CsCl density gradient (Figure 5), and must be limited so that CsCl precipitation is avoided (see RUN SPEEDS, above). The reference curves in Figure 5 show gradient distribution at equilibrium. Each curve in Figure 5 is within the density limits allowed for the TLA-100.4 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 5 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 curves in Figure 4.) Figure 5 can also be used to approximate the banding positions of sample particles.



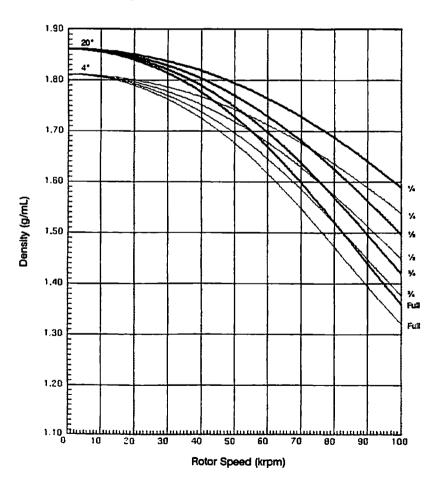
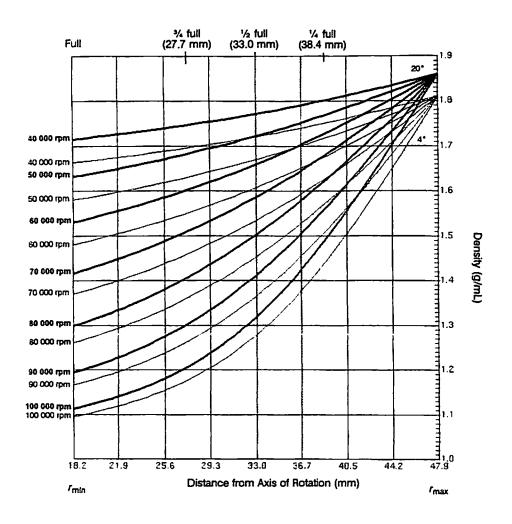


Figure 4. Precipitation Curves. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation of the TLA-100.4 rotor. Fill volumes are indicated on the curves. (These curves and the curves in Figure 5 were generated using 5.1-mL Quick-Seal tubes.)



حتث

Figure 5. CsCl Gradients at Equilibrium. Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 4) results in gradients presented here.

ADJUSTING FILL VOLUMES

Figures 4 and 5 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 *full* tube of a 1.55 g/mL homogeneous CsCl solution at 4°C may be centrifuged no faster than 70 000 rpm. Figure 5 presents the gradient profile for this speed and temperature: from 1.32 g/mL at the meniscus to 1.79 g/mL at the tube bottom. The same solution in a *three-quarter-filled* tube can be centrifuged at 76 000 rpm, in a *half-filled* tube at 84 000 rpm, and in a *quarter-filled* tube at 98 000 rpm. Gradient curves not presented in Figure 5 can be interpolated.

TYPICAL EXAMPLES FOR DETERMINING CsCI 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 4, find the curve that corresponds to the desired run temperature (20°C) and tube fill volume (full). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (76 000 rpm).
- 2. In Figure 5, sketch a horizontal line corresponding to each particle's buoyant 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 33.0 and 35.2 mm from the axis of rotation during centrifugation.

Example B: Knowing particle buoyant densities (e.g., 1.58 and 1.60 g/mL), how do you achieve good separation?

- 1. In Figure 5, sketch in a horizontal line corresponding to each particle's buoyant density.
- 2. Select the curve that gives good particle separation at the desired temperature (20°C). The tube volume required for this separation is shown on the horizontal axis.
- 3. Note the speed indicated along the curve (e.g., 60 000 rpm).
- 4. From Figure 4, determine the maximum allowable homogeneous CsCl density that corresponds to the selected temperature, speed, and fill volume from Figure 5.

In this example, particles will separate better along the 60 000 rpm curve than along the higher-speed curves. Particles will band at about 25.6 and 27.2 mm from the axis of rotation, about 1.6 mm of interband separation (center-to-center distance) at the rotor's 28-degree tube angle. When the tube is held upright, there will be about 2.8-mm interband separation. This interband distance, d, can be calculated from the formula:

$$d_{\rm up} = \frac{d_{\theta}}{\sin \theta}$$

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

CARE AND MAINTENANCE

MAINTENANCE

- Regularly apply silicone vacuum grease to the O-rings. Replace the O-rings about twice a year or whenever worn or damaged.
- Keep the threads of the rotor assembly lightly but evenly lubricated with Spinkote lubricant.

Store the rotor in a dry environment (not in the instrument) with the lid removed. Store the lid upside down to protect the plunger fingers from being damaged. If the plunger should become damaged, call your Beckman Field Service Representative regarding its repair or replacement. Refer to publication IN-175 for the chemical resistances of rotor and tube materials. Your Beckman Representative provides contact with the Field Rotor Inspection Program and the rotor repair center.

CLEANING

Wash the rotor occasionally to prevent buildup of residues. However, if salts or other corrosive materials are used or if spillage has occurred, wash the rotor and rotor components immediately. Do not allow corrosive materials to dry on the rotor. The Rotor Cleaning Kit (see the Supply List) contains two brushes and two quarts of a mild detergent, Beckman Solution 555 TM, for use with rotors and accessories. Dilute the detergent 5 or 10 to 1 with water. Rinse the cleaned rotor with distilled water and air-dry upside down. Lightly but evenly lubricate threads with Spinkote after washing.

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

DECONTAMINATION

If the rotor or tubes are contaminated with radioactive or pathogenic solutions, appropriate decontamination procedures should be followed. Check the chemical resistances list in publication IN-175 to be sure the decontamination method will not damage any part of the rotor.

STERILIZATION AND DISINFECTION

The rotor, including the O-rings, can be autoclaved at 121°C for up to an hour. Place the rotor in the autoclave upside down, with the lid removed. Ethanol (70%)¹ may be used on all rotor components, including those made of plastic.

While Beckman has tested these methods and found that they do not damage the rotor or components, no guarantee of sterility or disinfection is expressed or implied. When sterilization or disinfection is a concern, consult your laboratory safety officer regarding proper methods to use. 16 Miles

¹Flammability hazard. Do not use in or near operating ultracentrifuges.

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

Use the address label printed on the RGA form when mailing the rotor and/or accessories to:

Spinco Division Beckman Instruments, Inc. 1050 Page Mill Road Palo Alto, CA 94304

Attention: Returned Goods

SUPPLY LIST

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

REPLACEMENT ROTOR PARTS

Lid assembly	362312
O-ring (outer, rotor lid)	854519
O-ring (inner, rotor lid)	
Plunger assembly	349477

OTHER

Tubes and spacers	355872 345529 345530 349486
Adapter	
Quick-Seal tube sealer rack (for short tubes)	342423
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
Tube Topper rack (holds ½-in. tubes)	348122
Spacer removal tool (for 3.5-mL Quick-Seal tubes)	338765
Curved hemostat (6-in.)	927208
Fraction Recovery System Adapter Kit (for TL-series tubes)	347828
CentriTube Slicer	347960
Slicer blades (replacement pkg of 10)	348299
Adapter for ½-in. tubes	354526
Rotor Cleaning Kit	339558
Rotor cleaning brush	347404
Spinkote lubricant	306812
Silicone vacuum grease	335148
Solution 555	339555

Use one adapter per tube with rack part number 349486 when sealing 3.5-mL Quick-Seal tubes (part number 349621); use two when sealing 2.0-mL tubes (part number 345829).

SPECIAL TL-SERIES ROTOR WARRANTY

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Subject to the conditions specified below and the warranty clause of the Beckman terms and conditions of sale in effect at the time of sale, Beckman agrees to correct either by repair or, at its election by replacement, any defects of material or workmanship which develop within five (5) years after delivery of a TL-series rotor to the original Buyer by Beckman or by an authorized representative, provided that investigation and factory inspection by Beckman discloses that such defect developed under normal and proper use. Should a TL-series ultracentrifuge be damaged due to a failure of a rotor covered by this warranty, Beckman will supply free of charge all centrifuge parts required for repair.

Replacement

Any product claimed to be defective must, if requested by Beckman, be returned to the factory, transportation charges prepaid, and will be returned to Buyer with the transportation charges collect unless the product is found to be defective, in which case Beckman will pay all transportation charges.

A defective rotor will be replaced by Beckman at its then current list price less a credit based upon the age of the rotor (years since date of purchase). The Buyer shall not receive credit until the claimed defective rotor is returned to Beckman's Spinco Division at Palo Alto, California, or delivered to a Beckman Field Service representative.

The replacement price (cost to Buyer) for the respective rotor shall be calculated as follows:

Replacement price = current rotor list price x
$$\frac{\text{years}}{5}$$

Conditions

- 1. Except as otherwise specifically provided herein, this warranty covers the rotor only and Beckman shall not be liable for damage to accessories or ancillary supplies, including but not limited to (i) tubes, (ii) tube spacers, or (iii) tube contents.
- 2. This warranty is void if the rotor has been subjected to customer misuse such as operation or maintenance contrary to the instructions in the Beckman rotor or centrifuge manual.
- 3. This warranty is void if the rotor is operated with a rotor drive unit or in a centrifuge unmatched to the rotor characteristics, or is operated in a Beckman centrifuge that has been improperly disassembled, repaired, or modified.
- 4. Rotor bucket sets purchased concurrently with or subsequent to the purchase of a swinging bucket rotor are warranted only for term coextensive with that of the rotor for which the bucket sets were purchased.

Disclaimer

IT IS EXPRESSLY AGREED THAT THE ABOVE WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND OF THE WARRANTY OF MERCHANTABILITY AND THAT BECKMAN SHALL HAVE NO LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND WHATSOEVER ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE, OR REPLACEMENT OF THE PRODUCT.

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