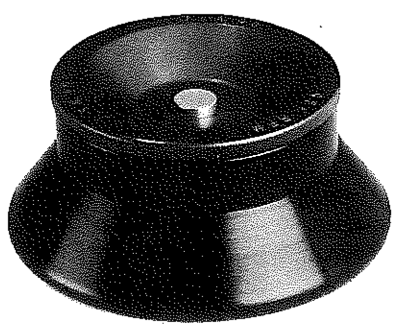
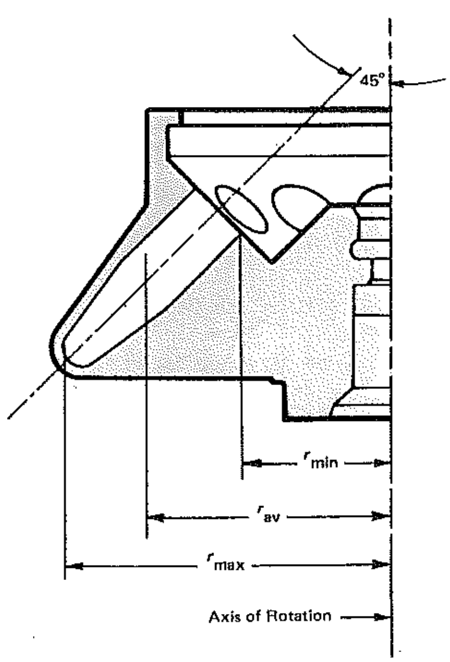
BECKMAN

INSTRUCTIONS FOR USING THE TLA-45 FIXED ANGLE ROTOR
in the Beckman TL-100 Tabletop Ultracentrifuge





U.S. Pat. No. 4,372,483
Japanese Pat. No. 1551443
Swiss Pat. No. 646,881

SPECIFICATIONS

Maximum speed .... 45 000 rpm
Density rating at full speed .... 1.7 g/mL
Relative Centrifugal Field* at maximum speed
 At r_{max} (55 mm) .... 125 000 x *g*
 At r_{av} (48 mm) .... 109 000 x *g*
 At r_{min} (25 mm) .... 56 700 x *g*
k factor at maximum speed 99
Number of tube cavities 12
Nominal dimensions of tubes .... 11 x 38 mm
Nominal tube capacity .... 1.5 mL
Nominal rotor capacity .... 18 mL
Approximate acceleration time to maximum speed
 (rotor fully loaded) .... 2 min
Approximate deceleration time from maximum speed
 (rotor fully loaded) .... 2 min
Weight of fully loaded rotor .... 0.8 kg (1.69 lb)
Rotor material .... aluminum
Conditions requiring speed reduction .... see Run Speeds

* 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:

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

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/s^2). After substitution:

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

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DESCRIPTION

The TLA-45 fixed angle rotor, rated for 45 000 rpm, holds up to twelve 1.5-mL microcentrifuge tubes at a 45-degree angle. Used in the TL-100 Tabletop Ultracentrifuge, this rotor develops sufficient centrifugal forces for a number of applications: it provides quick pelleting runs that preserve the bioactivity of a sample and it is an effective tool for the recovery of fine precipitates.

The rotor and lid (see Figure 1) are made of aluminum and are 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 twelve tube cavities are numbered to aid in sample identification.

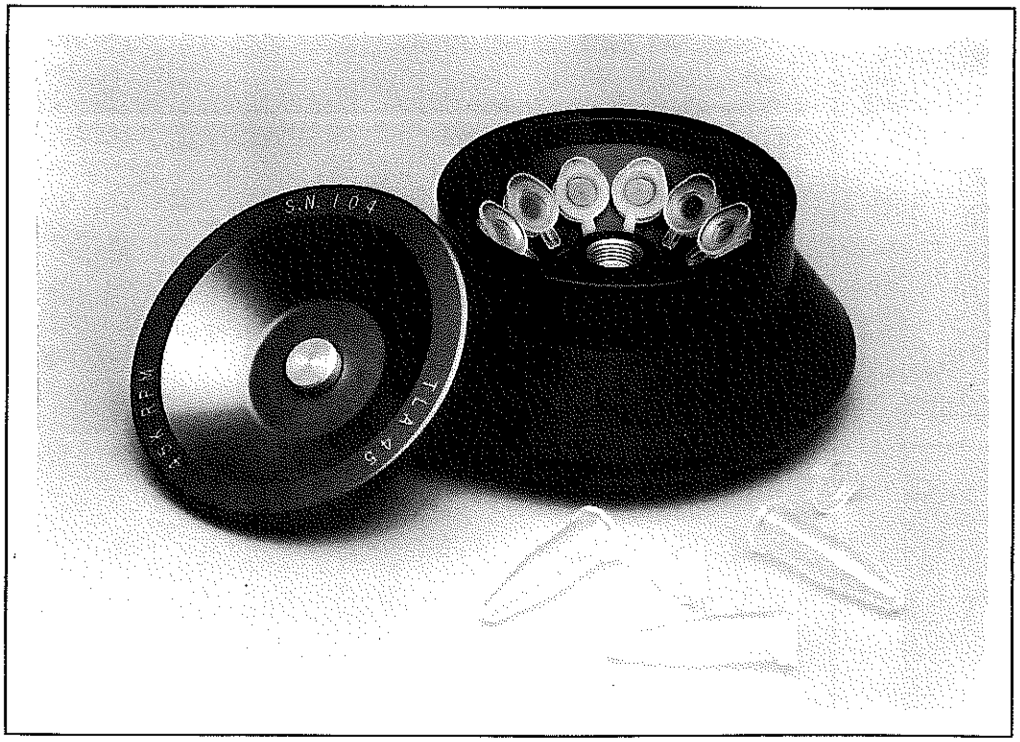

Figure 1. The TLA-45 Rotor with 1.5-mL Microfuge® Tubes.Each tube cavity is numbered to aid in sample identification.

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 TL-100 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. The TLA-45 rotor is warranted for five years (see the Warranty).

OPERATION

> **NOTE:** Specific information about the TLA-45 fixed angle rotor is given here. Use the TL-100 ultracentrifuge instruction manual (TL-IM) in combination with this bulletin for complete operating instructions.

TUBES

It is recommended that you use Beckman 1.5-mL Microfuge tubes in the TLA-45 rotor. These tubes are made of polyallomer and have attached caps and conical bottoms. They have been tested to withstand the high forces generated in this rotor at temperatures between 2 and 40°C.[1] Do not freeze polyallomer tubes before centrifugation, as they may become brittle and crack. These tubes are disposable and should be discarded after a single use. Consult publication IN-175 for additional information on the chemical resistances of rotor and tube materials.

Beckman cannot attest to the quality, durability, or strength of another manufacturer's microcentrifuge tubes. Additionally, another manufacturer's tubes may not be of the same dimensions and, if improperly fit in the rotor, could fail. If you use tubes made by another manufacturer, *Beckman highly recommends that you pretest these tubes using water samples* to determine the speed and *g*-forces the tubes can withstand.

ROTOR PREPARATION AND LOADING

Before using the rotor, lightly coat the metal threads with Spinkote™ lubricant and apply a thin film of silicone vacuum grease to the two O-rings in the lid. For runs at temperatures other than room temperature, refrigerate or warm the rotor beforehand.

Combinations of two, three, four, six, eight, nine and twelve tubes can be loaded into the TLA-45 rotor. *If you are using fewer than twelve tubes at a time, however, they must be arranged symmetrically around the center of the rotor.* Also, opposing tubes must be filled to the same level with liquid of the same density. After loading the tubes, place the lid on the rotor and tighten it firmly by hand.

ROTOR INSTALLATION

Use an absorbent towel to wipe off condensation from the rotor just before placing it in the TL-100. Then carefully install the rotor on the

[1] The maximum allowable speeds using Microfuge tubes in the TLA-45 rotor were determined using water samples. Use of other solvents may require that run speed be reduced to prevent tube failure.

drive hub and lock it in place as follows. Gently press 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 TL-100 instruction manual for complete information on operation.

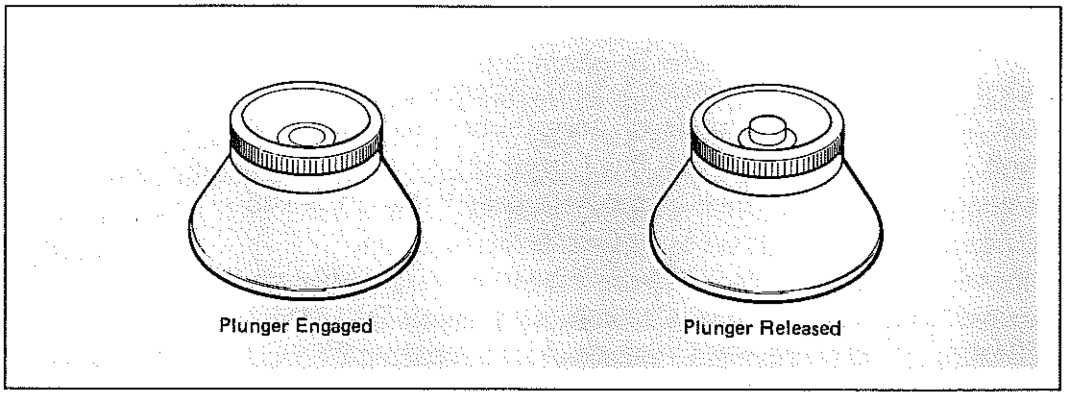

Figure 2. The Plunger in Locked and Released Positions in the Rotor Lid

ROTOR REMOVAL

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 original position. Remove the rotor from the instrument; then remove the rotor lid by unscrewing it counterclockwise by hand. Use a hemostat to remove the tubes from the rotor.

RUN TIMES

The k factor of the rotor (99 at maximum speed) is a measure of the rotor's pelleting efficiency. Use the k factor in the following equations to estimate run time t required to pellet particles of known sedimentation coefficient s (in Svedberg units).

$$t = \frac{k}{s} \qquad (1)$$

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

$$k = 99 \left(\frac{45\ 000 \text{ rpm}}{\text{actual run speed}} \right)^2 \qquad (2)$$

RUN SPEEDS

The centrifugal force at a given radius in a rotor is a function of run speed. Comparisons of forces between different rotors are made by comparing the rotors' relative centrifugal fields (RCF). When rotational speed is selected so that identical samples are subjected to the same RCF in two different rotors, one may then describe the samples as having been subjected to the same centrifugal force (refer to Table 1). You may need to reduce rotor speed when centrifuging high-density solutions or when running salts that may precipitate (e.g., CsCl).

Table 1. Relative Centrifugal Fields. Entries in this table are calculated from the formula RCF = 1.12 r (RPM/1000)² and then rounded to three significant digits.

Rotor Speed (rpm)	Relative Centrifugal Field (x *g*)			*k* Factor*
	At r_{max} (55 mm)	At r_{av} (48 mm)	At r_{min} (25 mm)	
45 000	125 000	109 000	56 700	99
40 000	98 600	86 000	44 800	125
35 000	75 500	65 900	34 300	163
30 000	55 400	48 400	25 200	222
25 000	38 500	33 600	17 500	320
20 000	24 600	21 500	11 200	499
15 000	13 900	12 100	6 300	888
10 000	6 160	5 380	2 800	1997

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

NOTE: The maximum allowable speeds using Microfuge tubes in this rotor were determined using water samples. Use of other solvents may require that run speed be reduced to prevent tube failure.

When centrifuging non-precipitating solutions with densities greater than 1.7 g/mL in this rotor, use equation (3) to calculate the maximum allowable rotor speed.

$$\text{RPM} = 45\ 000\ \text{rpm}\sqrt{\frac{1.7\ \text{g/mL}}{\text{density of tube contents}}} \quad (3)$$

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

When centrifuging CsCl or another self-forming-gradient salt, this equation will not usually guard against the precipitation of salt crystals. Figures 4 and 5, together with the description below, show how to reduce run speeds when using CsCl 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 or the rotor speed must be reduced. This prevents precipitation of salts concentrated at the tube bottom.

Solid CsCl has a density of 4 g/mL, and, if precipitated during centrifugation, may cause rotor damage or failure. Curves are provided up to the maximum rated speed of the rotor. Choose speed and CsCl density combinations that intersect on or below the curves shown in Figure 4 to ensure that CsCl will not precipitate during centrifugation. Curves are provided at two temperatures: 2°C (the black curves) and 4°C (the gray curves).

The curves in Figure 4 show that, at 20°C, tubes filled to any level can be run up to the maximum speed of the rotor if the CsCl density is 1.74 or less. At 4°C, Figure 4 shows that full tubes can be run up to the maximum speed of the rotor if the CsCl density is 1.67 or less; CsCl of density 1.71, for example, would require that speed be reduced to 39 000 rpm when using full tubes to avoid CsCl precipitation.

SELECTING CsCl GRADIENTS

Rotor speed determines the shape of a CsCl gradient (Figure 5) and must be limited so that CsCl precipitation is avoided (see RUN SPEEDS, above). The curves in Figure 5 show gradient profiles at *equilibrium*. Each curve was generated for a single rotor speed using the maximum allowable homogeneous CsCl densities (one for each fill level) that avoid precipitation at that speed and temperature.[2] Figure 5 can be used to approximate the banding positions of sample particles. Gradient curves not shown in Figure 5 can be interpolated.

MAINTENANCE

Do not use sharp tools on the rotor. 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, contact your Beckman Field Service Representative regarding its repair or replacement.

Routinely inspect the O-rings in the rotor lid. Replace them about twice a year or whenever they are worn or damaged. Apply silicone vacuum grease to the O-rings regularly and keep the metal threads of the rotor lightly lubricated with Spinkote lubricant. Refer to publication IN-175 for the chemical resistances of rotor and tube materials. Contact your Beckman Representative for information about the rotor repair center and the Field Rotor Inspection Program.

RADIOACTIVE DECONTAMINATION

A rotor (and/or accessories) contaminated with radioactive material should be decontaminated using a solution that will not damage its

[2] Gradients in Figure 5 result from homogeneous solutions, but can be more rapidly generated from step or linear gradients, as long as the total CsCl concentration is equal to the homogeneous solutions from the curves in Figure 4.

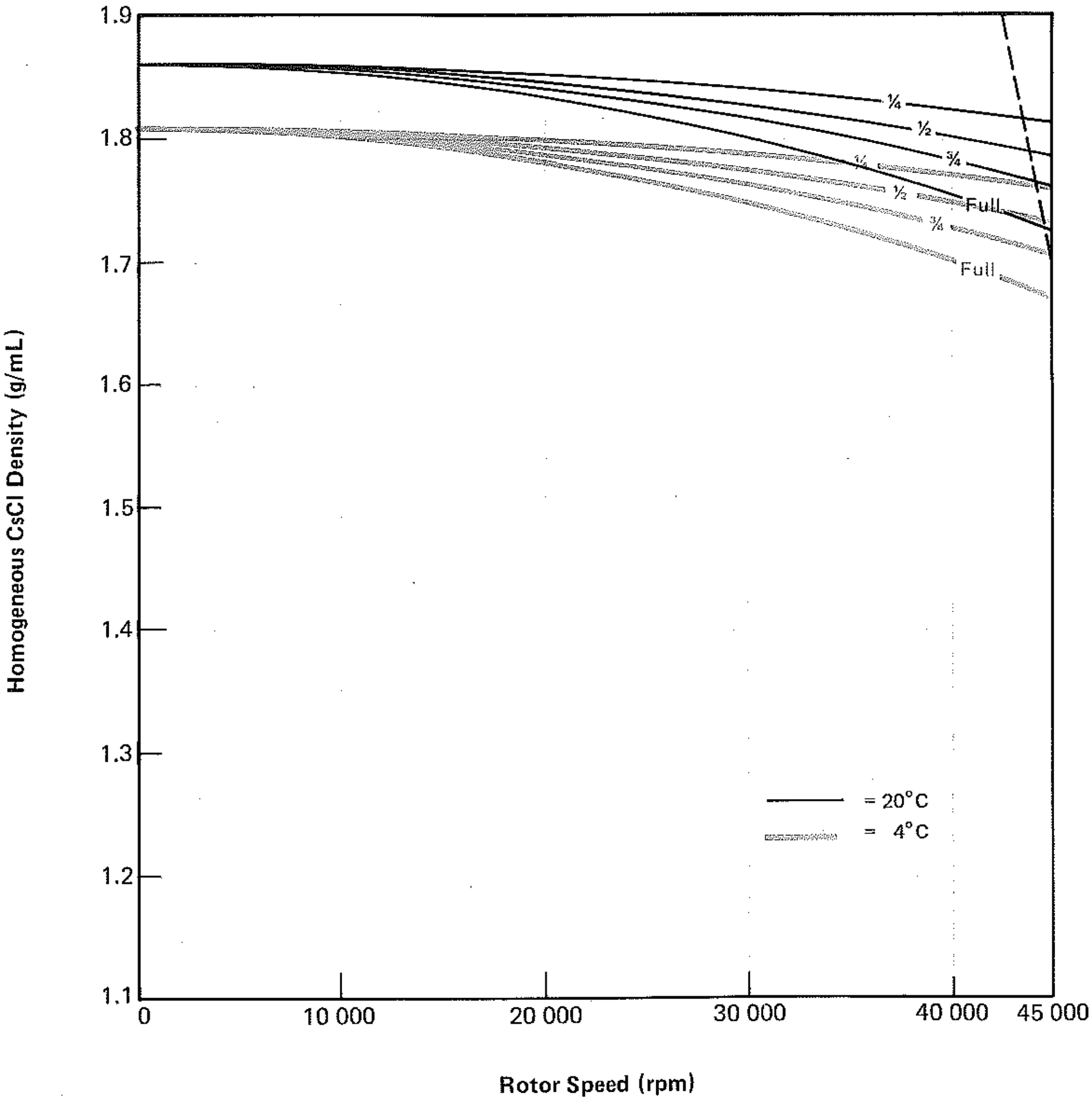

Figure 4. Precipitation Curves. Using combinations of rotor speeds and homogeneous CsCl solutions densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation. Tube fill levels are indicated on the curves. The dashed line is a representation of equation (3) and is shown here to illustrate the inability of that equation to guard against CsCl precipitation.

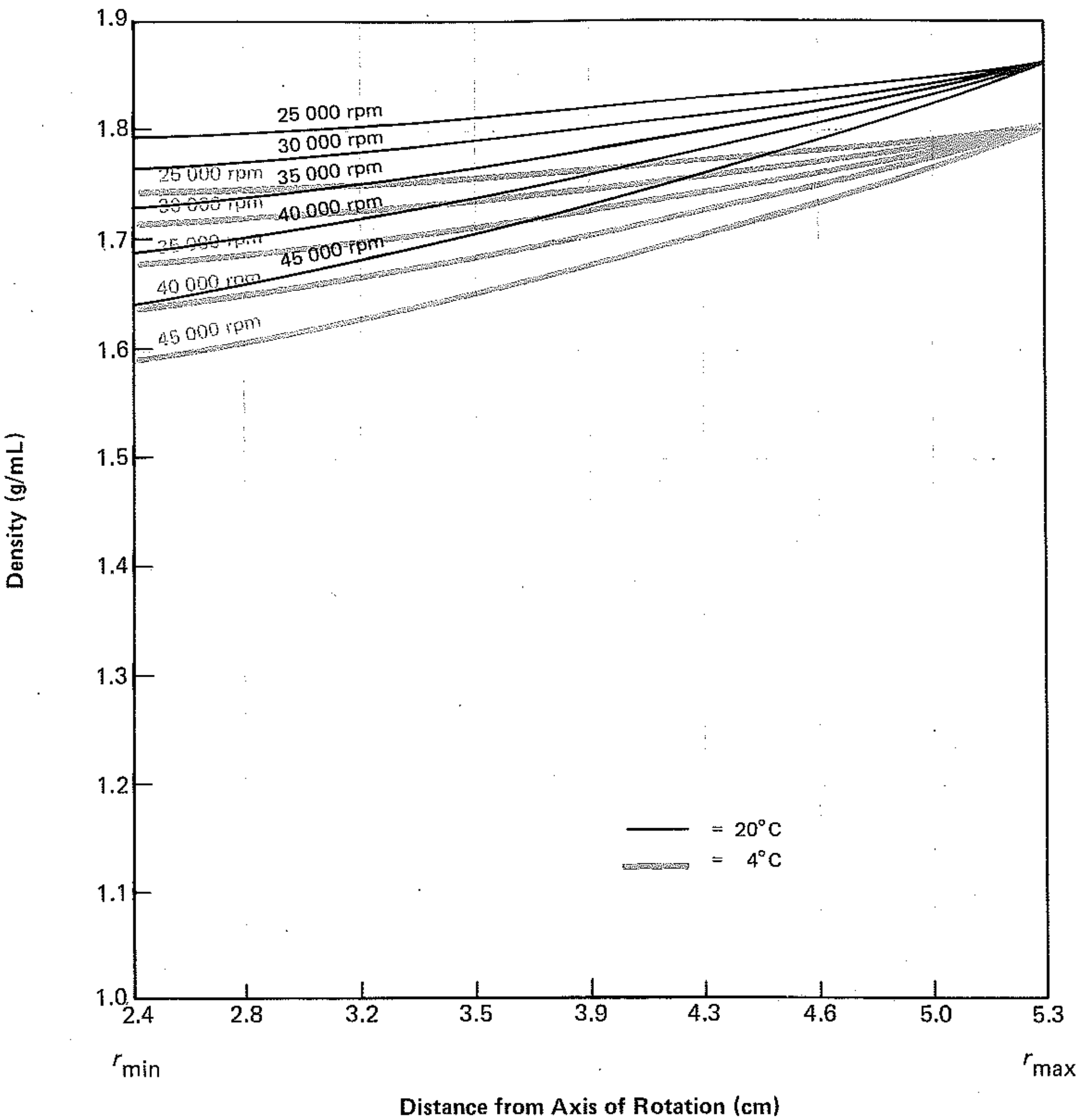

Figure 5. CsCl Gradients at Equilibrium. Density increases from the top to the bottom of the tube.

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 provided on the RGA form to mail the rotor or parts to:

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

Attention: Returned Goods

SUPPLY LIST

REPLACEMENT ROTOR SUPPLIES

TLA-45 rotor 358202
Lid assembly 358207
Lid O-ring (outer) 824644
Lid O-ring (inner) 824412
Rotor cleaning brush 347404

OTHER

1.5-mL Microfuge tubes (box of 500) 348349
Spinkote lubricant 306812
Silicone vacuum grease 335148
Rotor Cleaning Kit 339558
Solution 555 339555