TL-TB-006C
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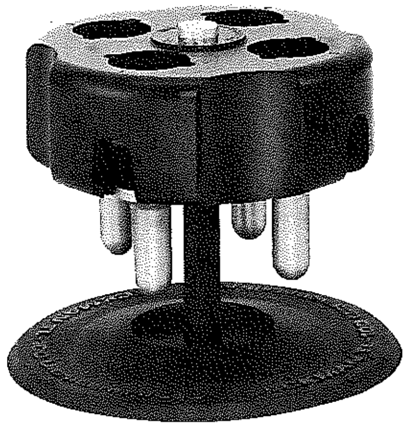
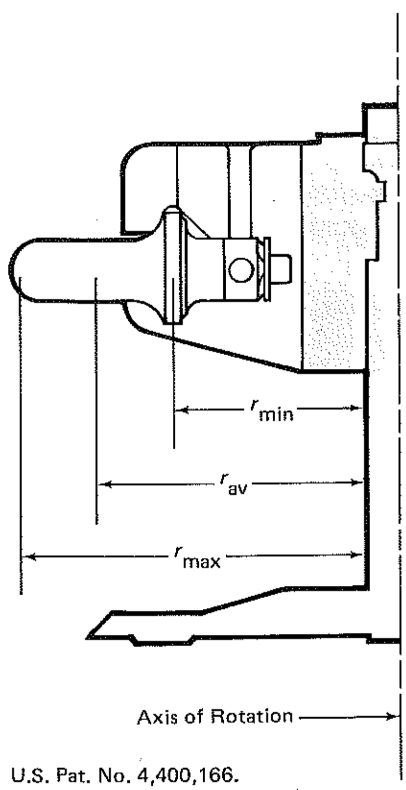
BECKMAN

INSTRUCTIONS FOR USING THE TLS-55 SWINGING BUCKET ROTOR

In the Beckman TL-100 Tabletop Ultracentrifuge





U.S. Pat. No. 4,400,166.

SPECIFICATIONS

Maximum speed	55 000 rpm
Density rating at full speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (76.5 mm)	259 000 x *g*
At r_{av} (59.4 mm)	201 000 x *g*
At r_{min} (42.2 mm)	143 000 x *g*
k factor at maximum speed	50
k' factors at maximum speed (5-20% sucrose, 5°C)	
When particle density = 1.3 g/mL	130
When particle density = 1.5 g/mL	119
When particle density = 1.7 g/mL	114
Number of buckets	4
Nominal tube dimensions	11 x 34 mm
Nominal tube capacity	2.2 mL
Nominal rotor capacity	8.8 mL
Available tubes	see Table 1
Approximate acceleration time to maximum speed (rotor loaded)	2 min
Approximate deceleration time from maximum speed (rotor loaded)	2½ min
Weight of fully loaded rotor	0.8 kg (1.8 lb)
Rotor body material	aluminum
Bucket 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 of radians per second (2πRPM/60), and g is the standard acceleration of gravity (9807 mm/s²). After substitution:

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

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DESCRIPTION

The TLS-55 swinging bucket rotor, rated for 55 000 rpm, holds four 11 x 34-mm ($\frac{7}{16}$ x 1$\frac{3}{8}$-in.) tubes. Used in the TL-100 Tabletop Ultracentrifuge, the rotor generates sufficient centrifugal forces for rate zonal banding of proteins, viruses, and DNA from small sample volumes.

The rotor body and bucket caps are made of aluminum and are black-anodized to resist corrosion. The buckets are titanium. O-rings made of Buna N rubber are located in the buckets to maintain atmospheric pressure. Buckets, bucket caps, and rotor body positions are numbered for your convenience. Bucket and cap assemblies are loaded from the top of the rotor; the titanium crossbars slide down into the slots provided in the rotor body. When not in the instrument, the rotor body must be supported on its rotor stand to permit the buckets to hang properly.

A plunger mechanism in the rotor body is used to lock the rotor in place on the drive hub before beginning the run. Engaging the plunger ensures that the rotor remains seated during centrifugation.

For overspeed protection, the TL-100 magnetically identifies the rotor's maximum rated speed during the run. This identification system includes a magnetic field sensor in the rotor chamber of the instrument and six magnets embedded in the bottom of the rotor. The overspeed system is designed to ensure that the rotor does not exceed its maximum permitted speed of 55 000 rpm.

The TLS-55 swinging bucket rotor is warranted for 5 years (see the Warranty).

OPERATION

NOTE: Specific information about the TLS-55 swinging bucket rotor is given here. Use the TL-100 instrument instruction manual (TL-IM) in combination with this bulletin for complete ultracentrifuge operation.

TUBES

Tubes for use in the TLS-55 rotor are listed in Table 1. Do not centrifuge any of the tubes below 2°C. Also, do not freeze polyallomer tubes before centrifugation, as they may become brittle and crack. Polycarbonate and polyallomer tubes may

Table 1. Available Tubes for the TLS-55 Swinging Bucket Rotor

Dimensions	Maximum Fill Volume (mL)	Description	Part Number	Spacer	Maximum Speed (rpm)	Operating Temperature Range (°C)
11 x 34 mm $\frac{7}{16}$ x 1$\frac{3}{8}$ in.	1.4	thickwall polycarbonate	343778*	none	55 000	2 - 40
	1.4	thickwall polyallomer	347287*	none	55 000	2 - 40
	2.2	thinwall polyallomer	347357†	none	55 000	2 - 40
	2.2	Ultra-Clear	347356†	none	55 000	4 - 20
11 x 32 mm $\frac{7}{16}$ x 1$\frac{1}{4}$ in.	2.0	Quick-Seal polyallomer	344625†	344674	55 000	2 - 40
11 x 25 mm $\frac{7}{16}$ x 1 in.	1.5	Quick-Seal polyallomer	344624†	344674	55 000	2 - 40

*Package of 100 †Package of 50

be centrifuged at temperatures above 25°C, but they should be pretested under anticipated run conditions. Ultra-Clear™ tubes, thinwall tubes made of thermoplastic, have been tested for use at temperatures between 4 and 20°C. For use at other temperatures, pretest these tubes as well under anticipated run conditions.

Thickwall polycarbonate tubes do not need caps. However, be sure to observe the maximum fill volumes listed in Table 1. Quick-Seal® tubes should be filled leaving a small air space at the base of the neck of the tube. These tubes do not need caps either, as they are heat sealed. Because of the small size of the tubes, adapters must be inserted into the Quick-Seal tube-sealing rack before beginning the heat-sealing process. Use the 344643 adapter when sealing the $\frac{7}{16}$ x 1-in. tubes; use the 344644 adapter when sealing the $\frac{7}{16}$ x 1$\frac{1}{4}$-in. tubes. Consult publications IN-163 and IN-180 for more detailed information on the use and care of Quick-Seal tubes. Consult publication IN-175 for additional information on the chemical resistances of rotor and tube materials

ROTOR PREPARATION

Before each use of the rotor, make sure that the bucket cap threads are lightly lubricated with Spinkote™ lubricant, and that the bucket O-rings are lightly coated with silicone vacuum grease. Never run a bucket without an O-ring, as it will leak. *For runs at other than room temperature, always refrigerate or warm the rotor to the desired temperature beforehand, since titanium is a poor conductor of heat.*

Opposing tubes must be filled to the same level (±0.1 mL) with liquid of the same density. It is important that you match numbered buckets with numbered caps, then screw caps into the buckets until there is metal-to-metal contact. Attach numbered bucket assemblies to corresponding rotor body positions as follows. Insert the bucket assembly into the rotor cavity; slide the crossbar down the grooves until seated in the slots (see Figure 1).

Two tubes can be run, if the buckets are attached to the rotor symmetrically, *and the two remaining empty buckets are also attached.* (If you run only *two* filled buckets regularly, alternate placement—positions 1 and 3, then 2 and 4—to ensure even wear of the rotor.)

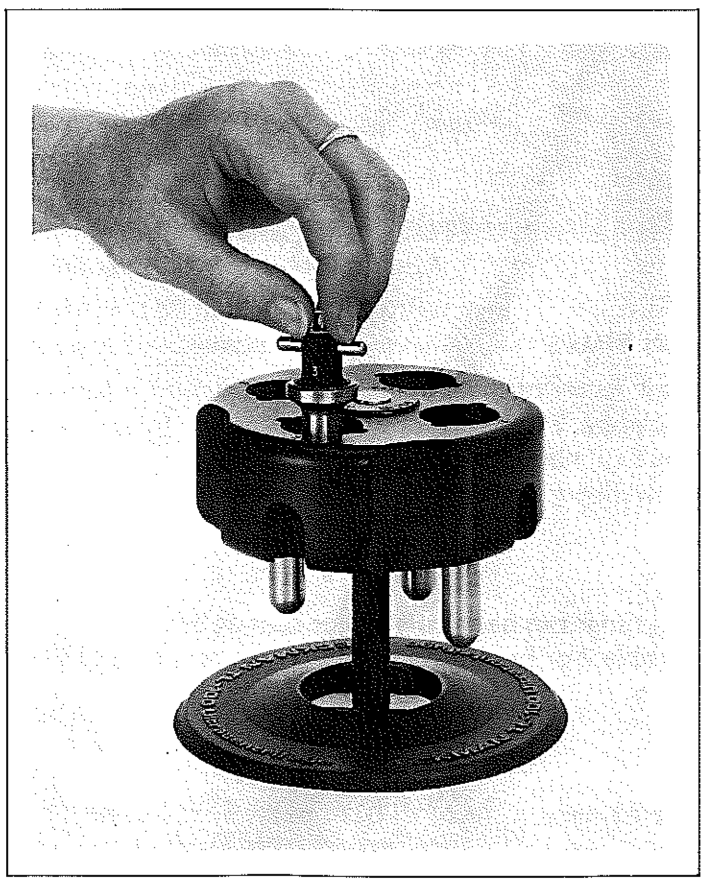

Figure 1. Attaching the Buckets onto the TLS-55 Rotor

NOTE: Remember, all four buckets must be attached to the rotor, whether loaded or empty. Attach them to the rotor body before installing it in the instrument. Trying to attach them after the rotor has been installed may cause damage to the drive shaft.

It is very important to lock the rotor in place before beginning the run.

Carefully place the rotor on the drive hub and lock it down as follows. Press the plunger on the rotor down until you hear a click. When you remove your finger, the plunger will remain flush with the rotor body (see Figure 2) if it is properly engaged. If the plunger pops up, repeat the procedure. Engaging the plunger ensures that the rotor remains seated during centrifugation. Consult the TL-100 instrument instruction manual for ultracentrifuge operation.

To release the plunger at the end of the run, press it down until you hear a click. When you remove your finger, the plunger will pop up to its original position (see Figure 2). Remove the rotor from the instrument and return it to its stand. Detach the buckets from the rotor body, unscrew the bucket caps, then use forceps or a hemostat to remove the tubes.

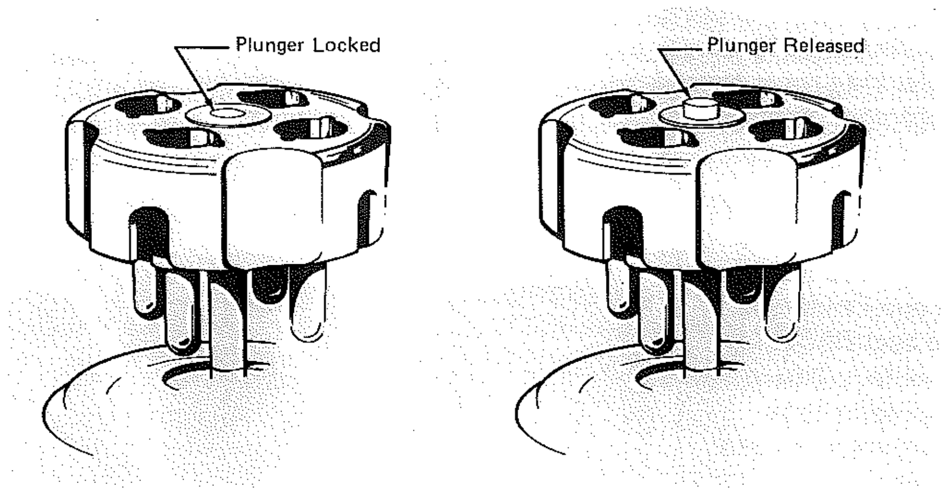

Figure 2. Locking and Releasing the Plunger

RUN TIMES

The k factor of the rotor (50 at 55 000 rpm) is a measure of the rotor's pelleting efficiency. The k factor can be used in the following equation to estimate the run time t (in hours) required to pellet particles of known sedimentation coefficient s (in Svedberg units).

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

Run times can be calculated for runs at less than maximum speed by using the k factors in Table 2 or by adjusting the k factor as shown below.

$$k = 50 \left(\frac{55\,000 \text{ rpm}}{\text{actual run speed}} \right)^2 \qquad (2)$$

Alternatively, k' factors (listed in the Specifications for various particle densities) can be used in the above equations to estimate the run time required to move a zone of particles of known sedimentation coefficient and density to the bottom of the gradient. Then, if the gradient is isokinetic, or nearly so, use that time to estimate the time required to move the zone a fraction of the distance of the tube length.

Equilibrium sedimentation (isopycnic gradient) run times should not be calculated using k or k' factors. CsCl gradients, for example, generally require at least overnight centrifugation for full tubes. Tubes partially filled with gradient solution will require less run time. Samples centrifuged at speeds slower than maximum allowable rates will require longer run times for good particle resolution.

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 being subjected to the same force (see Table 2).

Table 3. 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.*

Rotor Speed (rpm)	Relative Centrifugal Field (x g) At r_{max} (76.5 mm)	At r_{av} (59.4 mm)	At r_{min} (42.2 mm)	k Factor*
55 000	259 000	201 000	143 000	50
50 000	214 000	166 000	118 000	60
45 000	174 000	135 000	95 700	74
40 000	137 000	106 000	75 600	94
35 000	105 000	81 500	57 900	123
30 000	77 100	59 900	42 500	167

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

The maximum permitted speed for the TLS-55 rotor is 55 000 rpm. This speed may need to be reduced, however, under the following circumstances.

1. For centrifuging *nonprecipitating* solutions of densities greater than 1.7 g/mL, use the following square-root reduction formula to determine the allowable rotor speed.

$$RPM = 55\,000\sqrt{\frac{1.7\ g/mL}{\rho}} \qquad (3)$$

where ρ = density of tube contents

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

2. *When centrifuging CsCl or other self-forming-gradient salts, precipitation of salt crystals must be prevented.* 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 equilibrium. Figures 3 and 4, together with the description and samples below, show how to reduce run speeds when using CsCl gradients.

SELECTING CsCl GRADIENTS

Rotor speed is used to control the slope of a CsCl gradient, and must be limited so that CsCl precipitation is avoided (see RUN SPEEDS, above). Speed and density combinations that intersect on or below the curves in Figure 3 ensure that CsCl will not precipitate during centrifugation. The maximum allowable rotor speed depends on the temperature and the absolute amount of CsCl in the tube. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). For a given homogeneous CsCl solution density, the maximum allowable rotor speed increases as the fill volume decreases. For a given rotor speed, the allowable homogeneous CsCl solution density increases as the tube fill volume decreases.

The curves in Figure 4 show gradient distribution at equilibrium. Each curve in Figure 4 is within the density limits allowed for the TLS-55 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.[1] Figure 4 can also be used to approximate the banding positions of sample particles.

> **NOTE:** The curves in Figures 3 and 4 are for solutions of CsCl only. If other salts are present in significant concentrations, the overall CsCl concentration of salts must be reduced. This prevents precipitation of salts that concentrate at the tube bottom.

ADJUSTING FILL VOLUMES

Solutions may be centrifuged faster and for shorter run times when a tube is partially filled. If a tube is partially filled with gradient solution, mineral oil[2] (or some other low-density, immiscible liquid) must be floated on the tube contents to fill the tube to its maximum volume. Note, at a given speed, that as the fill level is decreased, the initial CsCl concentration required to generate the density distribution increases, according to Figure 3.

For example, a full tube of a 1.62 g/mL homogeneous CsCl solution can be centrifuged no faster than 44 000 rpm at 4°C (from Figure 3). The same solution in a ¾-filled tube can be centrifuged at 50 000 rpm, and Figure 4 shows the gradient generated, from 1.46 g/mL at the ¾-filled level to 1.81 g/mL at the tube bottom. In a ½-filled tube, a 1.62 g/mL CsCl solution can be centrifuged at 55 000 rpm.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS

Example A: Starting with a homogeneous CsCl solution density (e.g., 1.62 g/mL) and approximate particle densities (e.g., 1.59 and 1.61 g/mL), where will particles band?

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[1] The gradients in Figure 4 result from homogeneous solutions, but can be more rapidly generated from discontinuous gradients, as long as the total CsCl concentration is equal to the homogeneous solutions from the curves in Figure 3.

[2] Do not use an oil overlay in Ultra-Clear tubes.

1. In Figure 3, find the curve that corresponds to the desired run temperature (e.g., 20°C) and tube fill level (e.g., full). The rotor speed (from the abscissa) corresponding to the point where this curve and the homogeneous CsCl density intersect is the maximum allowable run speed (e.g., 50 000 rpm).
2. In Figure 4, sketch in a horizontal line corresponding to each particle density.
3. Mark the point in the figure where each particle density intersects the curve corresponding to the selected run speed (50 000 rpm) and temperature.
4. Particles will band at these locations along the tube length.

In this example, the 50 000 rpm curve shows that particles will band at about 17.8 and 16.3 mm from the bottom of the tube (r_{max})—about 1.5 mm apart. (To calculate the interband volume in milliliters, use $V = \pi r^2 h$, where r is the tube radius in centimeters, and h is the interband separation in centimeters. In this example, there will be about 0.14 mL of interband volume.)

Example B: Knowing particle densities (e.g., 1.49 and 1.52 g/mL), how do you get the best separation?

1. In Figure 4, sketch in a horizontal line corresponding to each particle density.
2. Select the curve at the desired temperature (e.g., 4°C) and tube volume (e.g., full) that gives the best particle separation.
3. Note the run speed indicated along the selected curve (e.g., 55 000 rpm).
4. From Figure 3, select the maximum homogeneous CsCl density (e.g., 1.52 g/mL) that corresponds to the temperature, run speed, and fill level established above. These parameters will provide the particle banding pattern selected in Figure 4.

In this example, particles of densities 1.49 and 1.52 g/mL will also separate along the 50 000 rpm curve, but it will take longer to generate the density gradient. At 55 000 rpm, bands will form at about 17.0 and 15.3 mm from r_{max} (i.e., 1.7 mm apart). Also, if a ¾-filled tube is used at 55 000 rpm, Figure 3 indicates that a 1.57 g/mL CsCl solution can be used to generate the gradient profile, and particles will band nearer to the top of the gradient.

MAINTENANCE

Do not use sharp tools on the rotor as they may scratch or damage the anodized surface. Store the rotor in a dry environment (not in the instrument) with bucket caps removed. Routinely inspect the bucket O-rings. Replace them about twice a year or whenever they are worn or damaged. Apply silicone vacuum grease to the O-rings before each run and keep the threads of the caps lubricated with Spinkote lubricant. (This is particularly important after cleaning the caps.) Contact your Beckman Representative for information on the Field Rotor Inspection Program and the rotor repair center.

CLEANING

If salt solutions or other corrosive materials have been run, or if spillage has occurred, wash the buckets immediately. Do not allow corrosive materials to accumulate on them. Use a mild detergent, such as Solution 555™ diluted 5 or 10 to 1 with water,

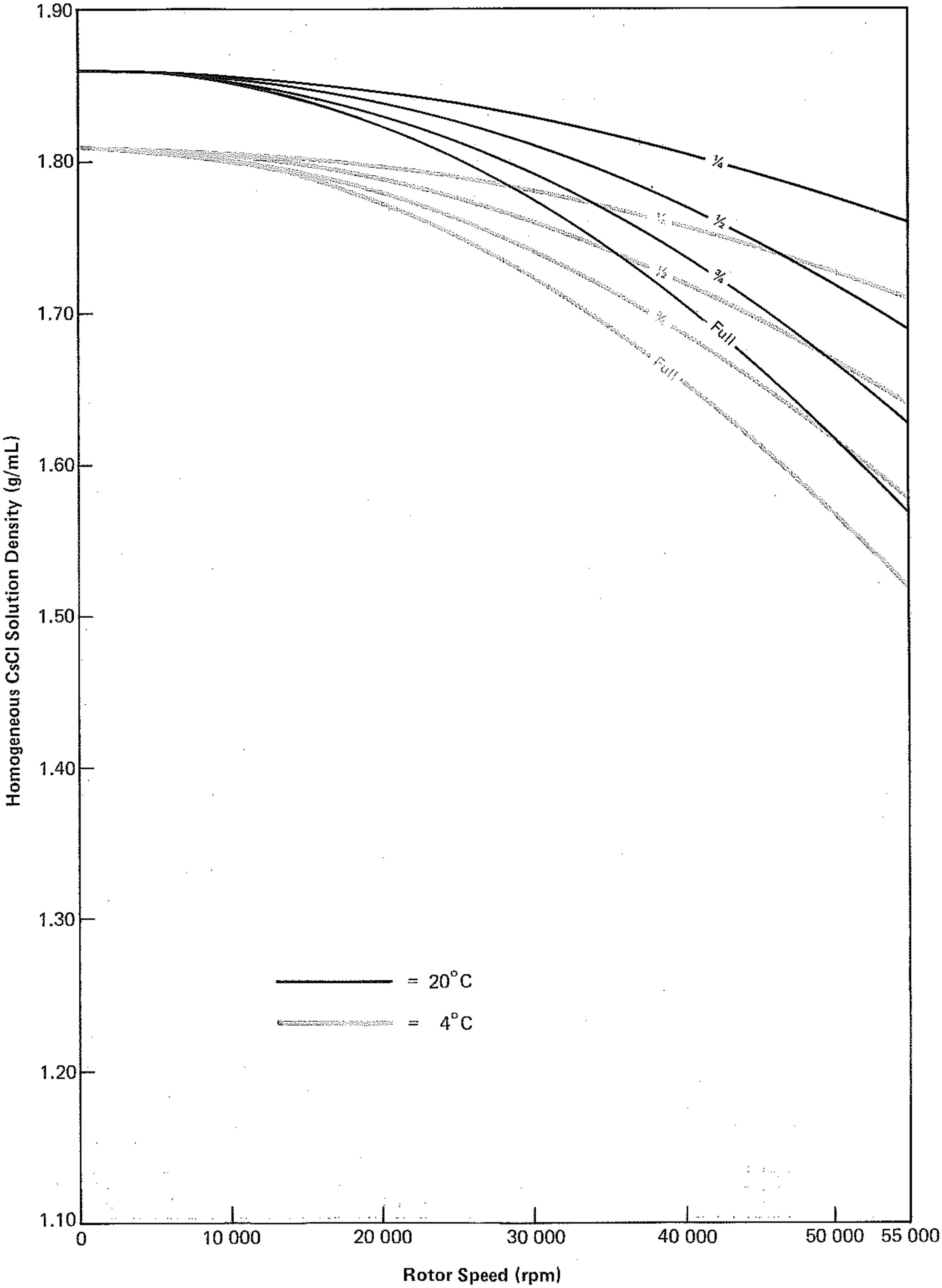

Figure 3. Precipitation Curves. Using density and speed combinations that intersect on or below the solid curves ensures that CsCl will not precipitate during centrifugation. Tube fill levels are indicated on the curves.

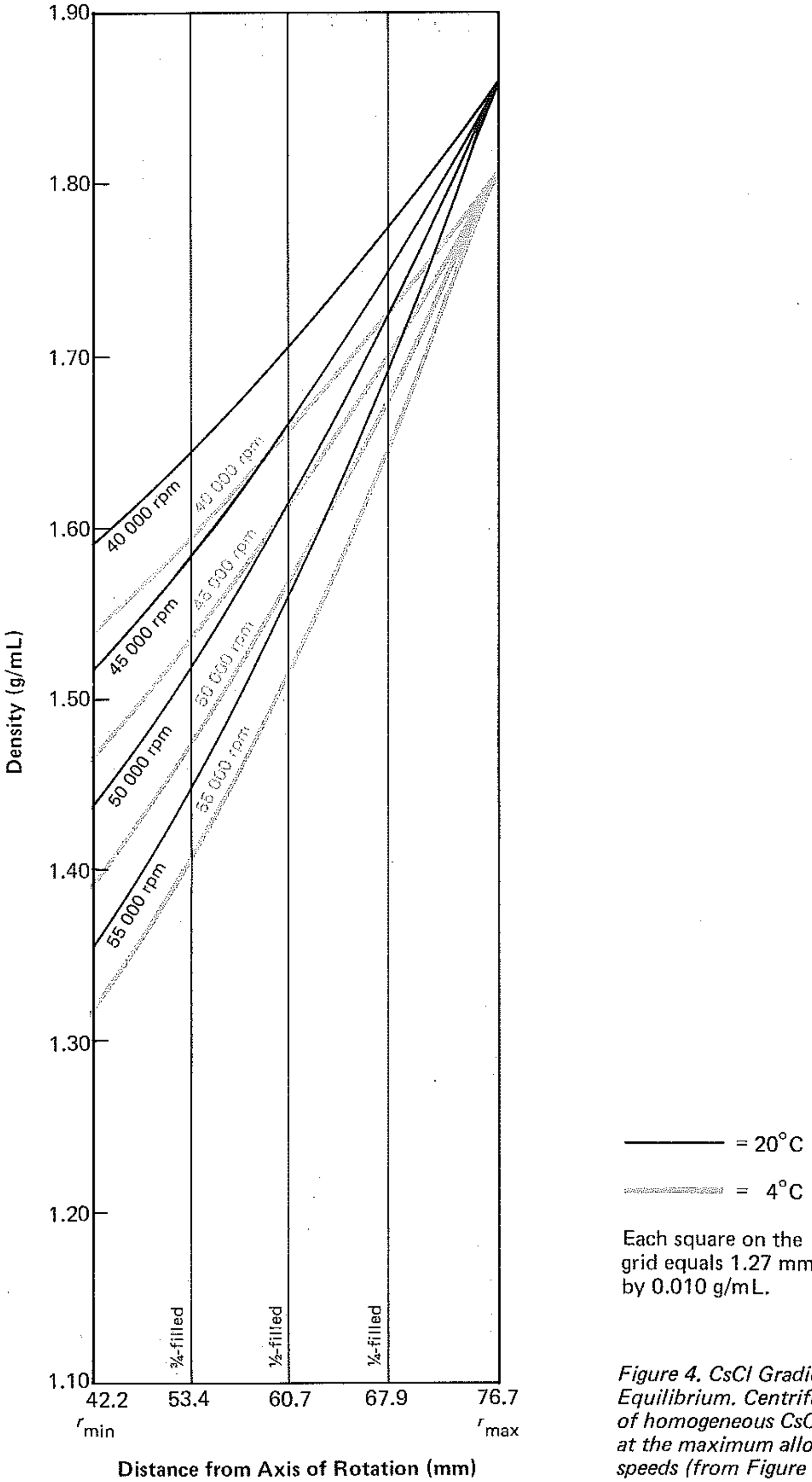

Figure 4. CsCl Gradients at Equilibrium. Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 3) results in the gradients presented. Density increases from the top to the bottom of the tube.

and a brush that will not scratch the buckets (see the SUPPLY LIST). Thoroughly rinse the cleaned buckets with water and air-dry them upside down. Bucket and cap threads should be cleaned several times a year. Clean the rotor body, including the hub cavity and bucket slots, periodically. DO NOT immerse the rotor body in water, however, since the plunger mechanism is difficult to dry and can rust. Be sure that all moisture is removed from any aluminum parts to avoid corrosion.

STERILIZATION

The rotor body and buckets, including the O-rings, can be sterilized by autoclaving at 121°C for an hour. Polycarbonate tubes can be autoclaved, although autoclaving reduces the usable lifetime of the tube. Use a cold sterilization method for polyallomer and Ultra-Clear tubes (see publication IN-175). The rotor and tubes can be disinfected with 70% ethanol.[3]

SUPPLY LIST

TLS-55 swinging bucket rotor	346936
Set of buckets	346364
Bucket cap	345770
Tubes	see Table 1
Bucket O-ring (4)	841648
Bucket stand	347358
Rotor stand	345773
Forceps	878446
Spinkote lubricant	306812
Silicone vacuum grease	335148
Rotor cleaning brush	347404
Beckman Solution 555	339555
Beckman CentriTube slicer kit	347960
Tube Topper kit (60 Hz)	348137
Tube Topper kit (50 Hz)	349647
Gold tube rack, 24 places	349387
Tube-sealing kit (with 60-Hz, 120V sealer)	345529
Tube-sealing kit (with 50-Hz, 220V sealer)	345530
Tube-sealer rack	342488

[3] Flammability hazard. Do not use in or near operating ultracentrifuges.