L5-TB-099
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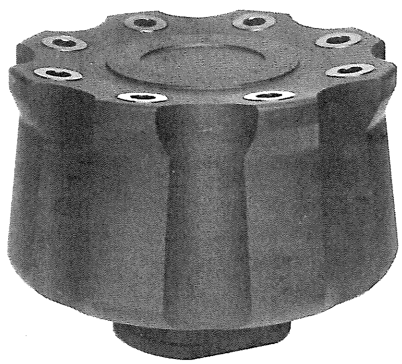
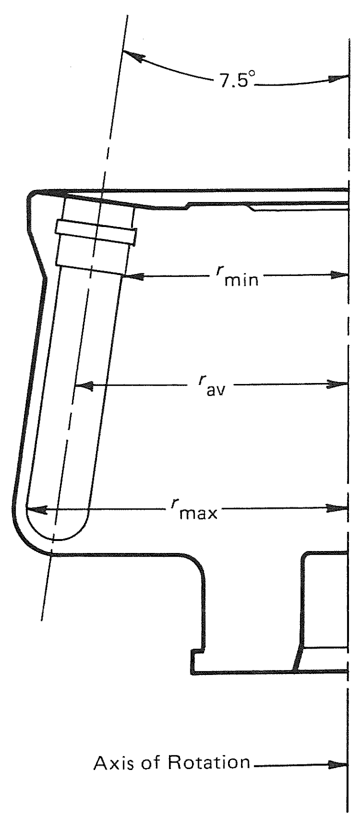
BECKMAN

INSTRUCTIONS FOR USING THE NVT 65 NEAR-VERTICAL TUBE ROTOR

In Beckman Class H, R, and S Preparative Ultracentrifuges





U.S. Pat. No. 4,102,490
U.S. Pat. No. 4,290,550
Japanese U.M. No. 1,469,154

SPECIFICATIONS

Maximum speed .... 65 000 rpm
Density rating at full speed .... 1.7 g/mL
Relative Centrifugal Field* at maximum speed
At r_{max} (84.9 mm) .... 402 000 x *g*
At r_{av} (72.2 mm) .... 342 000 x *g*
At r_{min} (59.5 mm) .... 282 000 x *g*
k factor at maximum speed 21
Number of tube cavities 8
Available tubes .... see Table 1
Nominal tube dimensions (largest tube) .... 16 x 76 mm ($^5/_8$ x 3 in.)
Nominal tube capacity .... see Table 1
Nominal rotor capacity .... 108 mL
Approximate acceleration time to maximum speed
(fully loaded, in an Optima XL ultracentrifuge) .... 10 min
Approximate deceleration time from maximum speed
(fully loaded, in an Optima XL ultracentrifuge) .... 7 min
Weight of fully loaded rotor .... 9.8 kg (22 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:

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

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 (9807mm/s^2). After substitution:

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

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DESCRIPTION

The NVT 65 rotor, rated for 65 000 rpm, is a near-vertical-tube rotor with a tube angle of 7.5 degrees. The rotor can centrifuge up to eight Quick-Seal® tubes and is used in class H, R, and S preparative ultracentrifuges. The slight angle of this rotor significantly reduces run times from a more conventional fixed angle rotor (with a tube angle of 20 to 30 degrees), while allowing components that do not band under separation conditions to either pellet to the bottom or float to the top of the tube.

One example of this type of sample is the separation of closed circular plasmid DNA from linear DNA in cesium chloride-ethidium bromide gradients. RNA will pellet; protein and other cellular components with low buoyant density in CsCl will float. The supercoiled plasmid will band in the central area of the tube with a starting CsCl density of approximately 1.55 g/mL. With typical sample loading, the plasmid band will not be in contact with the pellet or the components of the sample which float.

The rotor is made of titanium and is finished with black polyurethane paint. A hex-cavity rotor plug and tube spacer (see Figure 1) hold each tube in the rotor, and a plug gasket forms a closure around each plug. Rotor plugs are black-anodized aluminum, and spacers are blue-anodized aluminum. Because of the weight of the rotor, drive pins are not required in the rotor drive hub cavity.

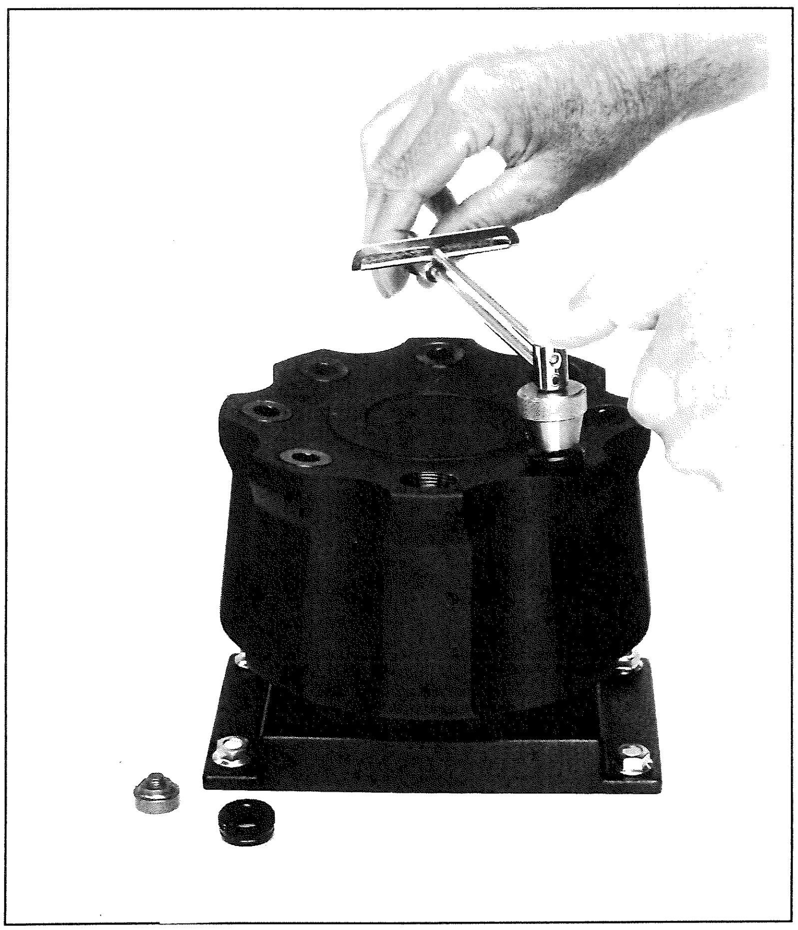

Figure 1. The NVT 65 Rotor and Required Accessories

A photoelectric detector in the ultracentrifuge monitors the overspeed disk on the bottom of the rotor and shuts down the run if speeds exceeding 65 000 rpm are detected.

The NVT 65 rotor is warranted for 5000 runs, 10 000 hours of centrifugation, or 5 years, whichever occurs first (see the Warranty).

PREPARATION AND USE

> **NOTE:** Specific information about the NVT 65 rotor is given here. Information common to this and other rotors is contained in the Rotors and Tubes for Preparative Ultracentrifuges Manual, LR-IM, which should be used together with this bulletin for complete rotor and accessory operation.

TUBES

The NVT 65 rotor uses Quick-Seal tubes listed in Table 1. Polyallomer tubes have been tested for use at temperatures between 2 and 25°C (at temperatures below 2°C polyallomer tubes may become brittle and lead to failure), and Ultra-Clear™ tubes at temperatures between 4 and 20°C. Before use at other temperatures, these tubes should be tested under simulated run conditions. Ultra-Clear tubes should not be autoclaved or used with solutions of pH greater than 8. See publication LR-IM for the care, cleaning, and chemical resistance of both kinds of Quick-Seal tubes and of rotor components. See the instruction bulletins on "How to Use Quick-Seal® Tubes" (IN-163 and IN-181) for filling and sealing the tubes.

The *g*-Max™ system uses a combination of short bell-top Quick-Seal tubes and floating spacers (also referred to as *g*-Max spacers). This system permits centrifuging the shorter tubes listed in Table 1 without reduction in *g* force. For more information on the *g*-Max system, see publication DS-709.

ROTOR PREPARATION

- Before using the rotor, make certain the overspeed disk is properly attached to the bottom of the rotor. If it is missing or has been damaged, do not run the rotor until you have replaced the disk (see the Supply List) according to instructions in publication LR-IM.

- Inspect the rotor plugs and gaskets for damage—the high forces generated in this rotor can cause damaged components to fail. Check that the plug threads are clean and lightly but evenly lubricated with Spinkote™ lubricant to ensure a proper seal by minimizing thread friction. For runs at other than room temperature, always refrigerate or warm the rotor to the required temperature beforehand, since titanium is a poor heat conductor.

> **NOTE:** Do not run an empty rotor. Place filled tubes in at least two opposing cavities. Make sure that cavities in use also have the proper floating spacers and/or spacers inserted before installing the rotor plugs.

Table 1. Available Quick-Seal Tubes for the NVT 65 Rotor. All may be centrifuged at 65 000 rpm.

Shape and Dimensions	Polyallomer Part Number (Box of 50)	Ultra-Clear Part Number (Box of 50)	Working Volume (mL)	Spacer Part Number	Floating Spacer* Part Number	Tube Rack† Part Number
Dome $^5/_8$ x 3 in. (16 x 76 mm)	342413	344322	13.5	349289	None	342488
Bell-Top $^5/_8$ x 2-$^5/_8$ in. (16 x 67 mm)	344622	—	10	349289	349901	344641
Bell-Top $^5/_8$ x 2-$^1/_4$ in. (16 x 58 mm)	344621	—	8	349289	356571	344640
Bell-Top $^5/_8$ x 1-$^3/_4$ in. (16 x 44 mm)	345830	—	6.3	349289	349900	344641 (with floating spacer 345828) ‡

* Floating spacers, part of the *g*-Max system of tube support, are made of Noryl, a registered trademark of General Electric.

† Standard tube sealer racks. If using the Tube Topper™, use tube rack 348123 (24 places), which is designed for all 5/8-in. diameter tubes.

‡ To seal 6.3-mL tubes in the standard sealer, first insert spacer 345828 upside-down in the rack.

Set the rotor into the vise, which should be bolted or clamped to a rigid surface. Load the filled and sealed tubes symmetrically into the rotor. Two, four, six, or eight tubes may be run if they are positioned in the rotor as shown in Figure 2. Tubes placed opposite each other must be filled with liquid of the same density. It is important that each cavity being used is completely filled. Therefore, if using the short bell-top tubes, place the correct floating spacer over the tubes. Place a spacer *on top of each tube or floating spacer* as shown in Figure 3. Then insert a rotor plug (gasket-end down) over each spacer and screw it in. Do not use rotor plugs in empty cavities. Using the hex plug adapter and torque wrench, tighten each rotor plug to 120 inch-pounds (13.6 N•m). The top surface of each rotor plug must be flush with the surface of the rotor. The rotor is now ready to be placed into an instrument for centrifugation.

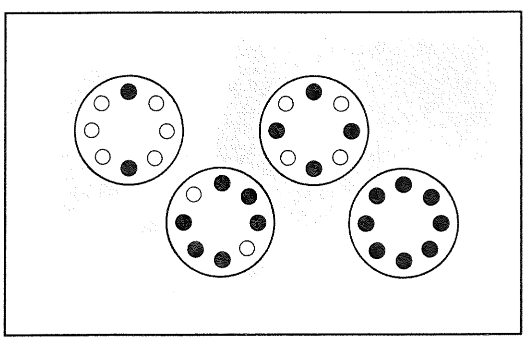

Figure 2. Arranging Tubes in the Rotor. Two, four, six, or eight tubes can be centrifuged per run, if they are arranged in the rotor as shown.

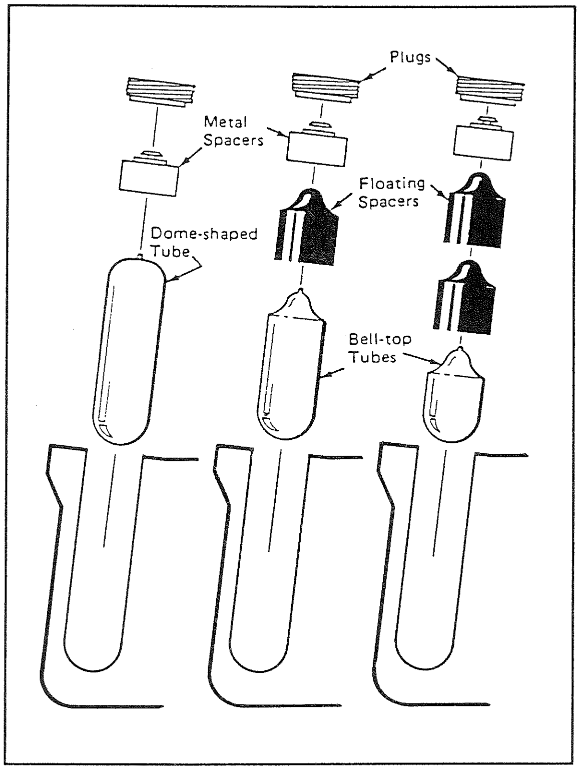

Figure 3. Correct Loading of the NVT 65 Rotor

OPERATION

Consult the appropriate instrument instruction manual for ultracentrifuge operation.

Vertical banding of sample and gradient formation occurs with centrifugation. With deceleration, tube contents reorient back to a horizontal position. For gradient stability when *preformed gradients* are used, select slow acceleration as follows:

- in the Optima XL and L8M series of ultracentrifuges, select a slow acceleration profile.
- in the Optima L series of ultracentrifuges, select SLOW ACCEL.
- in the L8 series of ultracentrifuges, select SLOW ACCEL ON (brake will be released at 750 rpm).
- in the L5 and L5B series ultracentrifuges without Slow Acceleration Accessories (SAA), set the acceleration dial on "1" (or SLOW) until the tachometer reads 2000 rpm. Then turn the dial to "10" (or FAST). For the stability of shallow preformed sucrose gradients of less than 5 to 20% (wt/wt), use of the SAA is recommended.
- in L5 and L5B series ultracentrifuges with SAA, turn the SAA on.

For the stability of *all gradients during deceleration,* do one of the following:

- in the Optima XL and L8M series of ultracentrifuges, select a slow deceleration profile.
- in the Optima L series of ultracentrifuges, select SLOW DECEL.
- in the L8 series of ultracentrifuges, select SLOW ACCEL ON (brake will drop out at 400 rpm).
- in the L7 ultracentrifuges, set the brake switch in the 800 rpm position.
- in the L5B series ultracentrifuges, set the BRAKE switch on SLOW or turn the SAA on (brake will drop out between 1500 and 1000 rpm).
- in L5 series ultracentrifuges that either have the SAA installed or that have been internally modified by a Beckman Service Representative, the brake will drop out between 1500 and 1000 rpm.
- in other L5 series ultracentrifuges, the brake may be turned off manually just as the rotor decelerates past 2000 rpm. Leaving the brake off from the beginning of the run is not recommended, as deceleration will take a long time.

REMOVAL

Remove the rotor from the instrument and return it to the rotor vise. Remove the plugs with the torque wrench and the spacers and tubes with the tube removal tool (part number 342419) provided. If floating spacers were used, remove them with the threaded end of the floating spacer removal tool (part number 338765).

CAUTION

If disassembly reveals evidence of leakage, and pathogenic or radioactive materials are involved, the operator should assume that some fluid escaped the rotor. Appropriate decontamination procedures should be applied to the centrifuge and accessories.

RUN TIMES

The *k* factor of the rotor is a measure of the rotor's pelleting efficiency. (Beckman has calculated *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 (ω = 0.105 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).

$$t = \frac{k}{s} \qquad (2)$$

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

$$k_{adj} = k \left(\frac{65\ 000}{\text{actual run speed}} \right)^2 \qquad (3)$$

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:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \qquad (4)$$

For more information on k factors see the Rotors and Tubes for Preparative Ultracentrifuges manual (LR-IM) and "Use of k Factor for Estimating Run Times from Previously Established Run Conditions" (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 is provided in Table 2.

Table 2. 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} (84.9 mm)	At r_{av} (72.2 mm)	At r_{min} (59.5 mm)	
65 000	402 000	342 000	282 000	21
60 000	342 000	291 000	240 000	25
55 000	288 000	245 000	202 000	30
50 000	238 000	202 000	167 000	36
45 000	193 000	164 000	135 000	44
40 000	152 000	129 000	107 000	56
35 000	116 000	99 100	81 600	74
30 000	85 600	72 800	60 000	100
25 000	59 400	50 500	41 700	144
20 000	38 000	32 300	26 700	225
15 000	21 400	18 200	15 000	400
10 000	9 510	8 090	6 660	900

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

If 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:

$$\text{reduced maximum speed} = 65\,000 \text{ rpm} \sqrt{\frac{1.7 \text{ g/mL}}{\text{density of tube contents}}} \qquad (5)$$

Further speed limits must be imposed when CsCl or other self-forming gradient salts are centrifuged, as equation (5) does not predict concentration limits that are required to avoid precipitation of salt crystals. Precipitation during centrifugation would alter the density distribution of CsCl and this would change sample separation. Figure 4, together with the description and examples below, shows how to reduce run speeds when using CsCl gradients.

SELECTING CsCl GRADIENTS

Figure 4 gives the CsCl density-limiting curves for full tubes. Figure 5 gives the equilibrium gradients that result from centrifugation using the maximum densities allowed by Figure 4 at several run speeds. Note that for a given rotor, the rotor speed will determine the slope of the gradient. At the lower speeds, longer run times will be required to achieve equilibrium.

> **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 may need to be reduced.

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 4, find the curve that corresponds to the desired run temperature (20°C). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (62 000 rpm). If the desired gradient curve is not presented in Figure 5, interpolate between the nearest curves and draw it in.

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 71.3 and 73.1 mm from the axis of rotation (about 1.8 mm of interband separation at the 7.5-degree tube angle).

When the tube is held upright, there will be about 13.8 mm of interband separation. This interband distance, *d*, can be calculated from the formula:

$$d_{up} = d_{\theta} / \sin \theta$$

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

Example B: Knowing particle densities (e.g., 1.640 and 1.670 g/mL), how do you get the best separation? Assume 20°C operation.

1. In Figure 5, sketch in a horizontal line corresponding to each particle's buoyant density.
2. Select the curve that gives the best particle separation at the desired temperature. Particles will band at points across the tube diameter where the sketched lines intersect this curve (lower axis) at equilibrium during centrifugation.
3. Note the run speed and homogeneous CsCl concentration for the selected curve.

CARE AND MAINTENANCE

MAINTENANCE

- Routinely inspect the overspeed disk on the bottom of the rotor. If it is damaged or missing, replace it (see publication LR-IM).

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

- The rotor plug gasket requires no maintenance except being kept clean. Replace if damaged. In general, sharp tools or brushes that may scratch the rotor surface should not be used. To replace the plug gasket, use the sharpened end of a cotton swab or similar nonmetallic tool to pry the gasket from the plug. *Do this carefully so that the plug is not damaged.* The new gasket snaps onto the grooved end of the plug.

Store the rotor in a dry environment (not in the instrument). Refer to publication LR-IM or publication IN-175 for the chemical resistances of rotor or 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 on the rotor. If salts or other corrosive materials have been run, or if spillage has occurred, wash the rotor, spacers, and rotor plugs immediately. Do not allow corrosive solutions to dry on the rotor or in plug threads. The Rotor Cleaning Kit (see the Supply List) contains two brushes that will not scratch and two quarts of mild Solution 555™ detergent for use with rotors, tubes, and accessories. Solution 555 should be diluted 5 or 10 to 1 with water. Rinse the cleaned rotor with distilled water and air-dry upside down. Relubricate with Spinkote after washing.

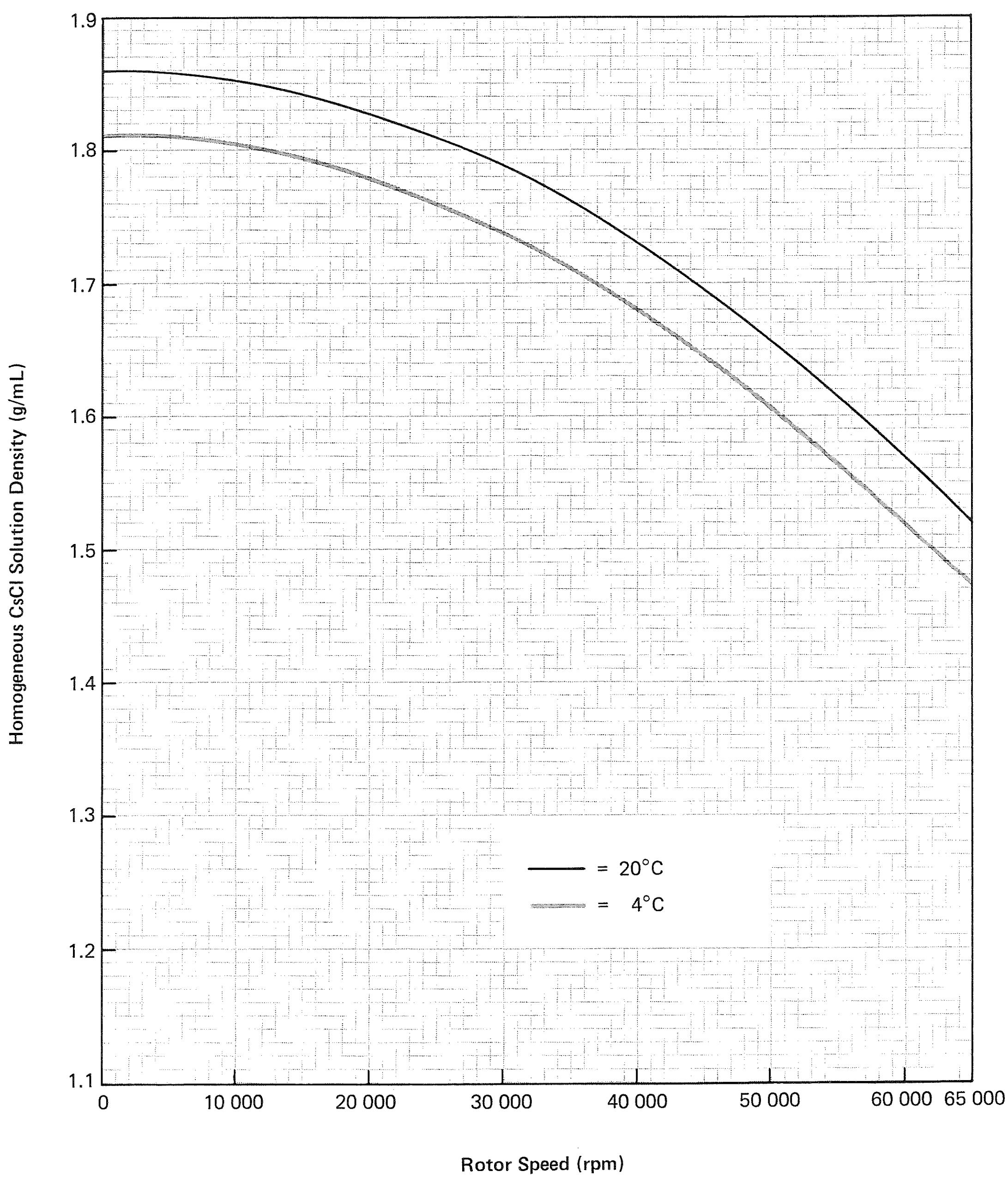

Figure 4. Precipitation Curves. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation. If gradient and sample solutions do not completely fill the tube, add mineral oil to fill. (Do not use an oil overlay in Ultra-Clear tubes.)

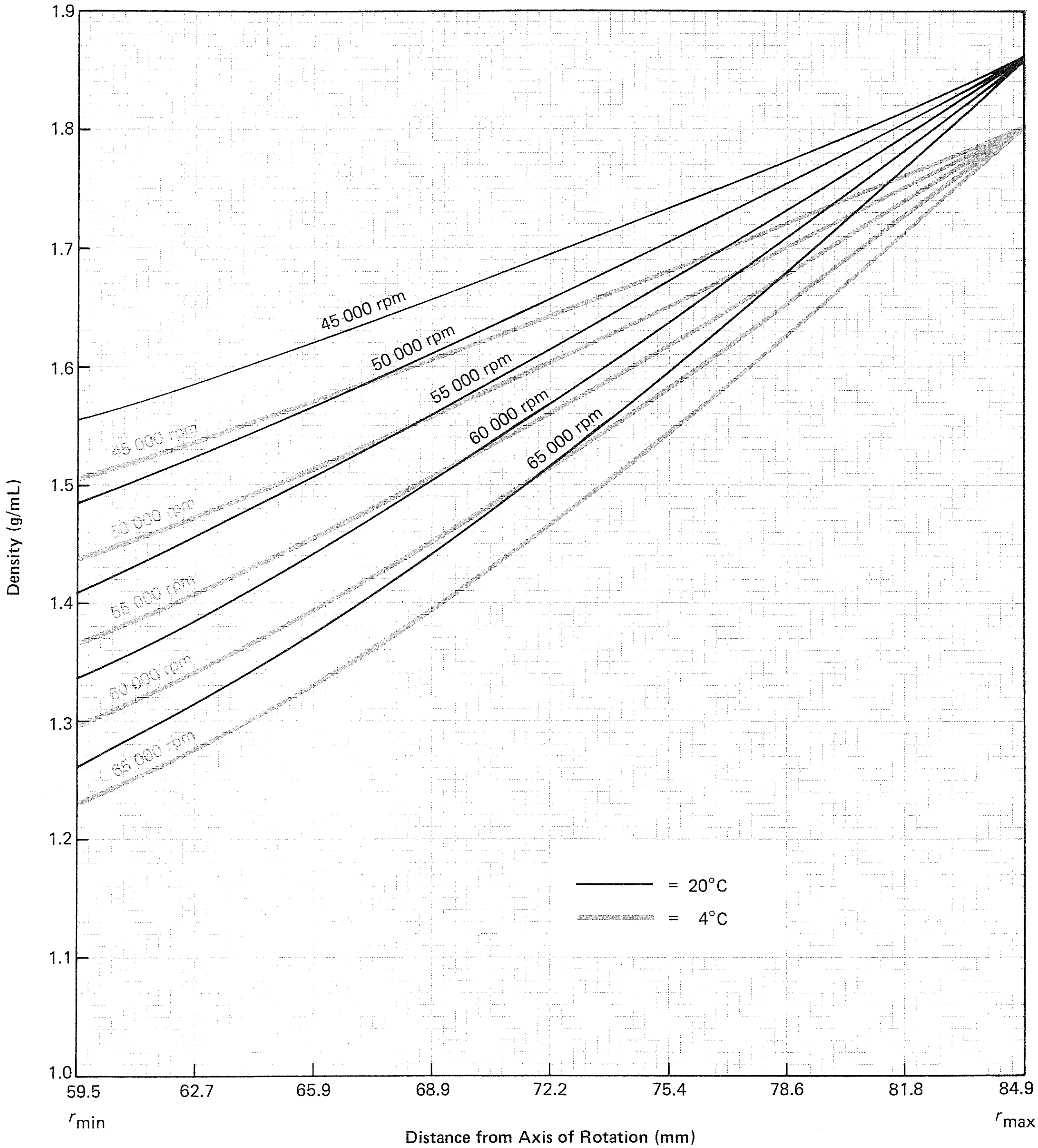

Figure 5. CsCl Gradients at Equilibrium. Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 4) results in gradients presented here. Density increases from the top (59.5 mm) to the bottom (84.9 mm) of the tube. (Black curves are for 20°C; gray curves are for 4°C.)

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 SUPPLIES

Rotor plug	355875
Rotor plug gasket	349290
Overspeed disk (65 000 rpm)	330338
Rotor vise assembly	342705

OTHER

Torque wrench	858121
Hex plug adapter	356306
Floating spacer removal tool	338765
Tube removal tool	342419
Sample application block	342694
Ultra-Clear tube sealing agent	345395
Spinkote lubricant	306812
Rotor cleaning kit	339558
Beckman Solution 555	339555
Quick-Seal tubes, spacers, floating spacers, racks	see Table 1
Tube sealing kit (with 60 Hz, 120 V sealer)	342429
Tube sealing kit (with 50 Hz, 220 V sealer)	342424
Quick-Seal Tube Topper (60 Hz)	348137
Quick-Seal Tube Topper (50 Hz)	349647
Beckman Fraction Recovery System	343890