

# TLS-55 Swinging-Bucket Rotor

For Use in the Beckman Coulter Optima MAX-XP, MAX-TL, MAX, MAX-E, TL, TLX, and TL-100 Tabletop Ultracentrifuges



TL-TB-006AT April 2020



Beckman Coulter, Inc. 250 S. Kraemer Blvd. Brea, CA 92821 U.S.A.



#### **TLS-55 Fixed Angle Rotor**

TL-TB-006AT (April 2020)

Copyright © 2020 Beckman Coulter, Inc. All rights reserved.

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

All other trademarks, service marks, products, or services are trademarks or registered trademarks of their respective holders.

#### **Contact Us**

If you have any questions, contact our Customer Support Center.

- Worldwide, find us via our website at www.beckman.com/support/technical.
- In the USA and Canada, call us at 1-800-369-0333.
- Outside of the USA and Canada, contact your local Beckman Coulter Representative.

Find us on the World Wide Web at: www.beckman.com

#### EC REP

Beckman Coulter Eurocenter S.A. 22, rue Juste-Olivier Case Postale 1044 CH - 1260 Nyon 1, Switzerland Tel: +41 (0) 22 365 36 11

May be covered by one or more pat. - see www.beckman.com/patents

Glossary of Symbols is available at beckman.com/ techdocs (PN C24689).

**Original Instructions** 

# **Revision History**

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released to the Beckman Coulter website. For labeling updates, go to www.beckman.com/techdocs and download the most recent manual or system help for your Beckman Coulter instrument, software, or accessory.

#### Revision AT, 4/2020

Updates were made to the following sections:

- Safety Notice
- Tubes and Accessories
- Supply List

**Revision History** 

# Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to use this equipment. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Beckman Coulter, Inc. urges its customers and employees to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other equipment.

## Alerts for Warning, Caution, and Note

#### 🕂 WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

#### **<u>A</u>** CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

**NOTE** NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

# **Safety Precautions**

#### **WARNING**

Risk of operator injury if:

- All doors, covers, and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broken parts.
- Doors, covers, and panels are not opened, closed, removed, and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers, and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers, and panels with care.
- Use the proper tools when troubleshooting.

#### 

Integrity could be compromised and operational failures could occur if this equipment is used in a manner other than specified. Use this rotor as instructed in this product manual.

#### CAUTION

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, contact us.

#### 

Risk of personal injury. Safety protection can be impaired if used in a manner not specified by the manufacturer. To avoid personal injury, use the rotor and instrument according to the manufacturer's instructions only.

## Safety Information for the TLS-55 Rotor



The safety information below summarizes information basic to the safe use of the rotor described in this manual. The international symbol displayed to the left is a reminder to the user that all safety instructions should be read and understood before operation or maintenance of this equipment is attempted. When you see the symbol on other pages of this publication, pay special attention to the safety information presented. Observance of safety precautions will also help to avoid actions that could damage or adversely affect the performance of the rotor.

Handle body fluids with care because they can transmit disease. No known test offers complete assurance that such fluids are free of micro-organisms. Some of the most virulent—Hepatitis (B and C) viruses, HIV (I–V), atypical mycobacteria, and certain systemic fungi—further emphasize the need for aerosol protection. Handle other infectious samples according to good laboratory procedures and methods to prevent spread of disease. Because spills may generate aerosols, observe proper safety precautions for aerosol containment.

Do not run toxic, pathogenic, or radioactive materials in this rotor without taking appropriate safety precautions. Biosafe containment should be used when Risk Group II materials (as identified in the World Health Organization *Laboratory Biosafety Manual*) are handled; materials of a higher group require more than one level of protection.

The rotor and accessories are not designed for use with materials capable of developing flammable or explosive vapors. Do not centrifuge such materials in nor handle or store them near the ultracentrifuge.

Although rotor components and accessories made by other manufacturers may fit in the TLS-55 rotor, their safety in this rotor cannot be ascertained by Beckman Coulter. Use of other manufacturers' components or accessories in the TLS-55 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.

Hook all four buckets, loaded or empty, to the rotor for every run. Make sure that filled containers are loaded symmetrically into the rotor and that opposing tubes are filled to the same level with liquid of the same density. Make sure that buckets containing Quick-Seal tubes have the proper floating spacers inserted (if applicable) before installing the bucket cap.

If disassembly reveals evidence of leakage, you should assume that some fluid escaped the rotor. Apply appropriate decontamination procedures to the centrifuge and accessories.

Never exceed the maximum rated speed of the rotor and labware in use. Refer to the section on *Run Speeds*.

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.

# Safety Data Sheets (SDS/MSDS)

To obtain an SDS for reagents used on Beckman Coulter centrifuges:

- **1.** On the internet, go to http://www.beckman.com:
  - a. Select Safety Data Sheets (SDS/MSDS) from the Support menu.
  - **b.** Follow the instructions on the screen.
  - c. Contact us if you have difficulty locating the information.
- 2. If you do not have internet access, contact us.

# Contents

#### Revision History, iii

Safety Notice, v Alerts for Warning, Caution, and Note, v Safety Precautions, vi Safety Information for the TLS-55 Rotor, vii Safety Data Sheets (SDS/MSDS), viii

#### TLS-55 Swinging Bucket Rotor, 1

Specifications, 1

Description, 2

Preparation and Use, 3 Prerun Safety Checks, 3 Rotor Preparation, 3 Operation, 5 Removal and Sample Recovery, 6 Tubes and Accessories, 6 Temperature Limits, 8 Quick-Seal Tubes, 8 Open-Top Tubes, 8 Run Times, 8 Run Speeds, 10

Selecting CsCl Gradients, 12 Adjusting Fill Volumes, 12 Typical Examples for Determining CsCl Run Parameters, 15

Care and Maintenance, 16 Maintenance, 16 Cleaning, 17 Decontamination, 17 Sterilization and Disinfection, 18 Storage, 18

#### Returning a Rotor, 18

Supply List, 19 Replacement Rotor Parts, 19 Other, 19

# Illustrations

1 Precipitat	ion Curves for the TLS-55 Rotor, 13
--------------	-------------------------------------

2 CsCl Gradients at Equilibrium for the TLS-55 Rotor, 14

# Tables

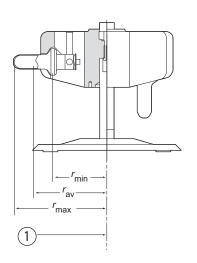
1	Beckman Coulter Tubes and Bottles for the TLS-55 Rotor, 7
2	Relative Centrifugal Fields for the TLS-55 Rotor, 11

Relative Centrifugal Fields for the TLS-55 Rotor, 11

Tables

# TLS-55 Swinging Bucket Rotor

# **Specifications**

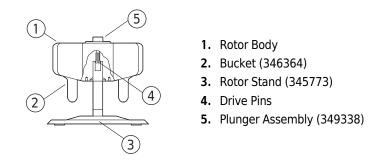


- U.S. Pat. No. 4,400,166
- 1. Axis of Rotation

Maximum speed         55,000 RPM
Density rating at maximum speed 1.7 g/mL
Relative Centrifugal Field <sup>a</sup> at maximum speedat $r_{max}$ (76.5 mm)
<i>k</i> factor at maximum speed 50
<pre>k' factors at maximum speed (5 to 20% sucrose gradient: 5°C) when particle density = 1.3 g/ml</pre>
Conditions requiring speed reductions see <i>Run Speeds</i>
Number of buckets 4
Available tubes see Table 1
Nominal tube dimensions (largest tube) $11 \times 34$ mm
Nominal tube capacity (largest tube) 2.2 mL
Nominal rotor capacity8.8 mL
Approximate acceleration time to maximum speed (fully loaded)
(fully loaded) 4½ min
Weight of fully loaded rotor.0.8 kg (1.69 lb)
Rotor body material aluminum
Bucket material titanium

a. 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 =  $r\omega^2/g$  — where r is the radius in millimeters,  $\omega$  is the angular velocity in radians per second (2  $\pi$  RPM /60), and g is the standard acceleration of gravity (9807 mm/s<sup>2</sup>). After substitution: RCF = 1.12r (RPM/1000)<sup>2</sup>

# Description



This Beckman Coulter rotor has been manufactured in an ISO 9001 or 13485 facility for use with the specified Beckman Coulter centrifuges.

The TLS-55 is a swinging-bucket rotor that holds four tubes. The TLS-55 rotor develops centrifugal forces that are suitable 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 for corrosion resistance. The buckets are titanium. Lubricated O-rings made of Buna-N rubber maintain atmospheric pressure inside the buckets during centrifugation. 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 locks the rotor to the drive hub before the run begins. Engaging the plunger ensures that the rotor remains seated during centrifugation.

The centrifuge identifies rotor 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 rotor has been tested<sup>\*</sup> to demonstrate containment of microbiological aerosols under normal conditions of the associated Beckman Coulter centrifuge when used and maintained as instructed.

Refer to the Warranty at the back of this manual for warranty information.

<sup>\*</sup> 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.

## **Preparation and Use**

Specific information about the TLS-55 rotor is given here. Information common to this and other rotors is contained in Rotors and Tubes for Tabletop Preparative Ultracentrifuges (publication TLR-IM-9), which should be used together with this manual for complete rotor and accessory operation.

**NOTE** Although rotor components and accessories made by other manufacturers may fit in the TLS-55 rotor, their safety in this rotor cannot be ascertained by Beckman Coulter. Use of other manufacturers' components or accessories in the TLS-55 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 section at the front of this manual before using the rotor.

- 1 Make sure that the rotor, buckets, and caps are clean and show no signs of corrosion or cracking.
- **2** Check the chemical compatibilities of all materials used (refer to *Chemical Resistances,* publication IN-175).
- **3** Verify that the tubes and accessories being used are listed in Table 1.

#### **Rotor Preparation**

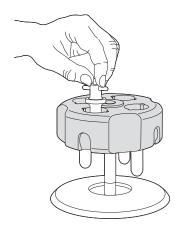
For runs at other than room temperature, refrigerate or warm the rotor beforehand for fast equilibration.

1 Before each use of the rotor, make sure that bucket cap threads are lightly but evenly lubricated with Spinkote lubricant (306812), and the bucket O-rings are lightly but evenly coated with silicone vacuum grease (335148).

**NOTE** Never run a bucket without an O-ring, as it will leak.

- **2** Dry the exterior of the filled tubes (see page 6 for tube information), then slide them into the buckets.
  - (Moisture between the bucket and the tube can cause tube collapse and create resistance to tube extraction after centrifugation.)
  - All opposing tubes for a run must be filled to the same level ( $\pm 0.1 \text{ mL}$ ) with liquid of the same density.

- **3** Use the required adapters or floating spacers, if required, to complete the loading operation.
- **4** Match numbered caps with numbered buckets.
  - **a.** Screw the caps into the buckets until there is metal-to-metal contact.
  - **NOTE** Two tubes can be run if the filled buckets are attached in opposing positions on the rotor (positions 1 and 3, or 2 and 4), and the two remaining buckets are also attached. (If you regularly run only two filled buckets, alternate the placement—positions 1 and 3, then 2 and 4—to ensure even wear on the rotor.)
- **5** Attach numbered bucket assemblies to corresponding rotor body positions.
  - **a.** Insert the bucket assembly into the rotor cavity.
  - **b.** Attach all buckets, loaded or empty.
- ${\bf 6} \quad {\rm Slide \ the \ crossbar \ down \ the \ grooves \ until \ it \ is \ seated \ in \ the \ slots}$



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

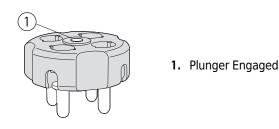
#### Operation

Refer to *Rotors and Tubes* (publication TLR-IM-9) for information on installing swinging bucket rotors.

**1** To install the rotor, carefully lift it with both hands and place it 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.



**a.** If the plunger pops up, repeat the procedure.

#### 

In all ultracentrifuge models except the Optima MAX-TL, MAX-XP, MAX, 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.

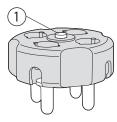
- **4** For additional operating information, see the following:
  - *Run Times*, page 8, for using *k* factors to adjust run durations.
  - *Run Speeds*, page 10, for information about speed limitations.
  - *Selecting CsCl Gradients*, page 12, for methods to avoid CsCl precipitation during centrifugation.

#### **Removal and Sample Recovery**

## 

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.



1. Plunger Released

• When you remove your finger the plunger will pop up to its released position.

**2** Remove the rotor from the ultracentrifuge and return it to its stand.

**3** Detach the buckets from the rotor body.

**4** Unscrew the bucket caps, then use forceps or a hemostat to remove the tubes.

#### **Tubes and Accessories**

The TLS-55 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* (publication TLR-IM-9) for information on the chemical resistances of tube and accessory materials.

Tube		Required Accessory			
Dimensions and Max Volume	Description	Part Number	Description	Part Number	Max Speed/ RCF/ <i>k</i> Factor
11 × 34 mm 2.2 mL	Ultra Clear	347356 (pkg/50)	none	_	55,000 RPM 259,000 × <i>g</i> 50
11 × 34 mm 2.2 mL	thinwall polypropylene	347357 (pkg/50)	none	_	55,000 RPM 259,000 × g 50
13 × 32 mm 2.0 mL	Quick-Seal polypropylene	344625 (pkg/50)	Noryl <sup>b</sup> floating spacer	344674	55,000 RPM 259,000 × <i>g</i> 50
11 × 25 mm 1.5 mL	Quick-Seal polypropylene	344624 (pkg/50)	Noryl floating spacer	344674	55,000 RPM 259,000 × g 37
11 × 34 mm 1.4 mL	thickwall polypropylene	347287 (pkg/100)	none	_	55,000 RPM 259,000 × <i>g</i> 50
11 × 34 mm 1.4 mL	thickwall polycarbonate	343778 (pkg/100)	none	_	55,000 RPM 259,000 × <i>g</i> 50
7 × 20 mm 230 μL	thickwall polycarbonate	343775 (pkg/100)	Delrin <sup>c</sup> adapter	358615 (pkg/4)	55,000 RPM 249,000 × <i>g</i> 23
7 × 20 mm 230 μL	thickwall cellulose propionate	342303 (pkg/100)	Delrin adapter	358615 (pkg/4)	55,000 RPM 249,000 × <i>g</i> 23
5 × 20 mm 175 μL	thinwall polypropylene	342630 (pkg/100)	Delrin adapter	358614 (pkg/4)	55,000 RPM 248,000 × g 22
5 × 20 mm 175 μL	thinwall cellulose propionate	342630 (pkg/100)	Delrin adapter	358614 (pkg/4)	55,000 RPM 248,000 × g 22
5 × 20 mm 175 μL	thinwall polyethylene	343622 (pkg/100)	Delrin adapter	358614 (pkg/4)	55,000 RPM 248,000 × <i>g</i> 22

Table 1 Beckman Coulter Tubes and Bottles for the TLS-55 Rotor<sup>a</sup>

a. Use only the items listed here.

b. Noryl is a registered trademark of GE Plastics.

c. Delrin is a registered trademark of E.I. DuPont de Nemours & Company.

# 25°C

#### **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 buckets.

- 1 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* (publication TLR-IM-9) for detailed information on the use and care of Quick-Seal tubes.

Some of the tubes listed in Table 1 are part of the *g*-Max system. The *g*-Max 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 tubes listed in the table in the TLS-55 rotor without reduction in g force. Additional information about the *g*-Max system is available in publication DS-709.



a-Max

Spacer

#### **Open-Top Tubes**

Open-top tubes should be filled as full as possible for tube support. If necessary, float mineral oil (or some other low-density, immiscible liquid) on top of the tube contents to fill the tube to its maximum volume. (Do not use an oil overlay in Ultra-Clear tubes.)

#### **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:

EQ 1

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

where  $\omega$  is the angular velocity of the rotor in radians per second ( $\omega = 0.105 \times \text{RPM}$ ),  $r_{\text{max}}$  is the maximum radius, and  $r_{\text{min}}$  is the minimum radius.

1-9

After substitution:

TL-TB-006AT

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).

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

 $k_{adj} = k \left(\frac{55,000}{actual run speed}\right)^2$ 

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:

 $\frac{t_a}{t_b} = \frac{k_a}{k_b}$ 

For more information on k factors see Use of k Factor for Estimating Run Times from Previously Established Run Conditions (publication DS-719).

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

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

EQ 4

EQ 2

EQ 3

EQ 5

#### **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 are subjected to the same force. The RCF at a number of rotor speeds is provided in Table 2.

Do not select rotational speeds in excess of 55,000 RPM. In addition, 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:

EQ 6

reduced maximum speed = (55,000 RPM) 
$$\sqrt{\frac{1.7 \text{ g/mL}}{\rho}}$$

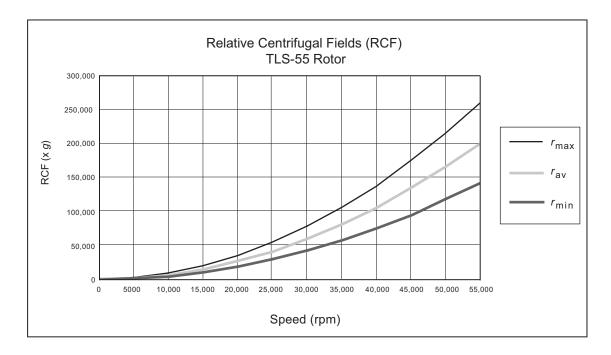
where  $\rho$  is the density of the tube contents. This speed reduction will protect the rotor from excessive stresses due to the added tube load. *Note, however, that the use of this formula may still produce maximum speed values that are higher than the limitations imposed by the use of certain tubes 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. Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation may cause rotor failure. Figure 1 and Figure 2, together with the description and examples below, show how to reduce run speeds when using CsCl gradients.

	Relativ			
Rotor Speed (RPM)	At r <sub>max</sub> (76.5 mm)	At r <sub>av</sub> (59.4 mm)	At r <sub>min</sub> (42.2 mm)	<i>k</i> 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	116
30,000	77,100	59,900	42,500	123
25,000	53,600	41,500	29,500	167
20,000	34,300	26,600	18,900	341
10,000	19,300	15,000	10,600	377
10,000	8,570	6,650	4,730	690

<b>T</b> 1 1 A	B 1 11	<u> </u>			
lable 2	Relative	Centrifugal	Fields for	the IL:	S-55 Rotor <sup>a</sup>

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



# **Selecting CsCl Gradients**

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 1 ensure that CsCl will not precipitate during centrifugation in the TLS-55 rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). Curves in Figure 1 and Figure 2 are provided up to the maximum rated speed of the rotor.

**NOTE** The curves in Figure 1 and Figure 2 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.

The reference curves in Figure 2 show gradient distribution at equilibrium. Each curve in Figure 2 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. (The gradients in Figure 2 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 2.) Figure 2 can also be used to approximate the banding positions of sample particles. Curves not shown in the figure may be interpolated.

#### **Adjusting Fill Volumes**

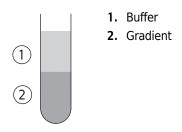


Figure 1 and Figure 2 show that several fill volumes are possible in a tube. If a thinwall tube is partially filled with gradient solution, float mineral oil (or some other low-density, immiscible liquid) on top of the tube contents to fill the tube to its maximum volume. (Do not use an oil overlay in Ultra-Clear tubes.) 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 *half-filled* tube of 1.62-g/mL homogeneous CsCl solution at 4°C may be centrifuged at 55,000 RPM (see Figure 1). The segment of the 55,000-RPM curve (Figure 2) from the half-filled line to the tube bottom represents this gradient. The same solution in a *three-quarter-filled* tube may be centrifuged no faster than 50,000 RPM. A tube full of the 1.62-g/mL CsCl solution may be centrifuged no faster than 44,000 RPM (curves not shown in the figure may be interpolated).

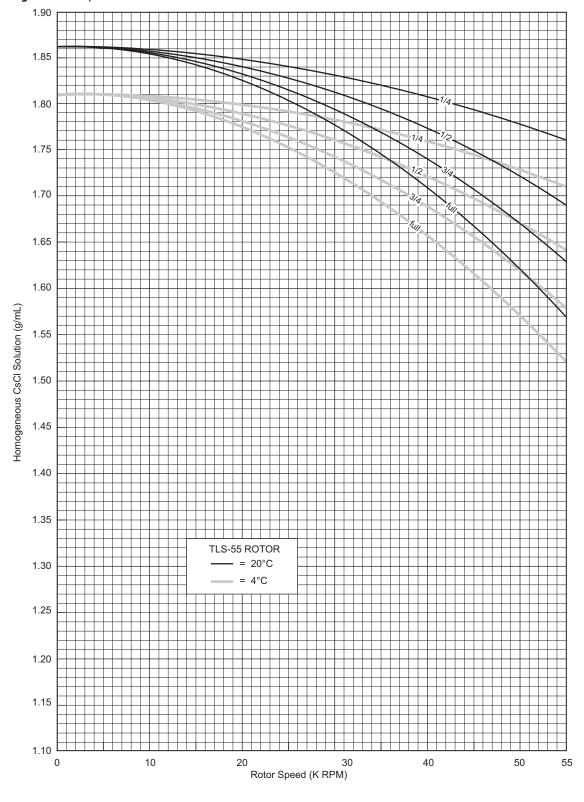


Figure 1 Precipitation Curves for the TLS-55 Rotor\*

<sup>\*</sup> 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.

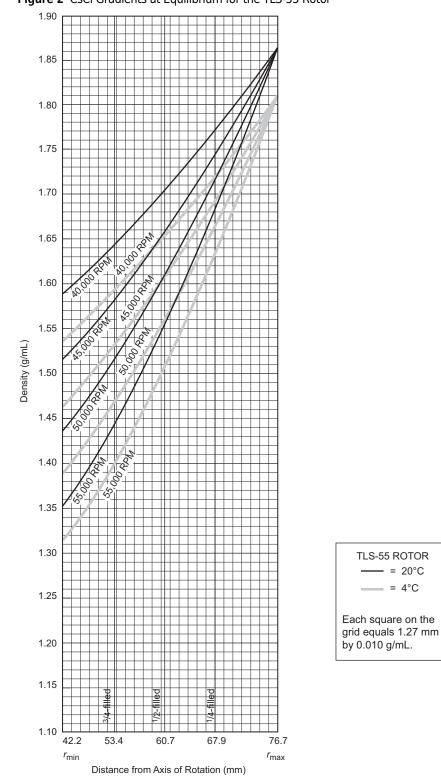


Figure 2 CsCl Gradients at Equilibrium for the TLS-55 Rotor\*

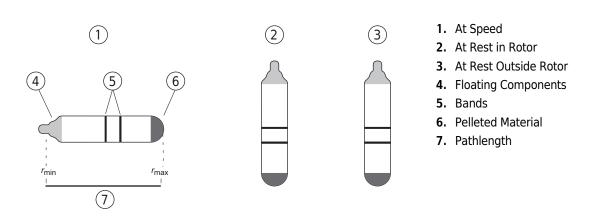
<sup>\*</sup> Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 1) results in gradients presented here.

#### **Typical Examples for Determining CsCl Run Parameters**

#### Example A:

Starting with a homogeneous CsCl solution density of 1.62 g/mL and approximate particle buoyant densities of 1.59 and 1.61 g/mL, at 20°C, where will particles band at equilibrium?

- In Figure 1, find the curve that corresponds to the desired 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 (50,000 RPM).
- 2 In Figure 2, 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 17.8 and 16.3 mm from the tube bottom ( $r_{max}$ ), about 1.5 mm of centerband-to-centerband separation.

To determine interband volume in milliliters, use the following equation:

EQ 7

 $V = \pi r^2 h$ 

where *r* is the tube radius in centimeters and h is the interband separation in centimeters.

#### Example B:

Knowing particle buoyant densities (for example, 1.49 and 1.52 g/mL), how do you achieve good separation?

- 1 In Figure 2, sketch in a horizontal line corresponding to each particle's buoyant density.
- **2** Select the curve at the desired temperature (4°C) and tube volume (full) that gives the best particle separation.
- **3** Note the run speed along the selected curve (55,000 RPM).
- **4** From Figure 1, 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.

In this example, particles will band at about 17.0 and 15.3 mm from the tube bottom (about 1.7 mm apart).

## **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.

- **1** Frequently check the bucket O-rings (841648) for signs of wear.
  - a. Replace O-rings every 6 months, or whenever worn or damaged.
  - **b.** Keep the O-rings lightly coated with silicone vacuum grease (335148).
- **2** Regularly lubricate the bucket cap threads with a thin, even coat of Spinkote lubricant (306812) before every run.

Refer to *Appendix A* in *Rotors and Tubes* (publication TLR-IM-9) 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 Wash the rotor buckets, O-rings, and caps in a mild detergent, such as Beckman Solution 555, that won't damage the rotor.
  - The Rotor Cleaning Kit contains two plastic-coated brushes and two quarts of Solution 555 (339555) for use with rotors and accessories.
  - Dilute the detergent 10 to 1 with water.
- **2** Wash the rotor body with a sponge or cloth dampened with a mild detergent, such as Solution 555, diluted 10 to 1 with water.

**NOTE** Do not immerse the rotor body in water, since the hanger mechanism is difficult to dry and can rust.

- **3** Rinse the cleaned rotor and components with distilled water.
- **4** Air-dry the buckets upside down.
  - Do not use acetone to dry the rotor.
- **5** Clean metal threads frequently to prevent buildup of residues and ensure adequate closure.
  - **a.** Use a brush and concentrated Solution 555.
  - **b.** Rinse and dry thoroughly, then lubricate lightly but evenly with Spinkote to coat all threads.

#### 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* (publication TLR-IM-9) to select an agent that will not damage the rotor

## **Sterilization and Disinfection**

- The rotor and all rotor components and accessories except for those made of Noryl<sup>\*</sup> can be autoclaved at 121°C for up to an hour. Remove the lids from the rotor buckets and place the rotor, buckets, lid, and spacers in the autoclave upside down.
- Ethanol (70%)<sup>†</sup> or hydrogen peroxide (6%) may be used on all rotor components, including those made of plastic. Bleach (sodium hypochlorite) may be used, but may cause discoloration of anodized surfaces. Use the minimum immersion time for each solution, per laboratory standards.

While Beckman Coulter 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.

Refer to publication IN-192 (included with each box of tubes) for tube sterilization and disinfection procedures. *Quick-Seal, Ultra Clear, and thinwall open-top tubes are disposable and should be discarded after a single use.* 

#### Storage

121°C

When it is not in use, store the rotor in a dry environment (not in the instrument) with the bucket lids removed to allow air circulation so moisture will not collect in the tube cavities.

## **Returning a Rotor**

Before returning a rotor or accessory for any reason, prior permission must be obtained from Beckman Coulter, Inc. This form may be obtained from your local Beckman Coulter sales office. The form, entitled *Returned Material Authorization* (RMA) for United States returns or *Returned Goods Authorization* (RGA) for international returns, should contain the following information:

- rotor type and 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 email address of the person to be notified upon receipt of the rotor or accessory at the factory,
- name and email address of the person to be notified about repair costs, etc.

To protect our personnel, it is the customer's responsibility to ensure that all 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.

<sup>\*</sup> Noryl is a registered trademark of GE Plastics.

<sup>†</sup> Flammability hazard. Do not use in or near operating ultracentrifuges.

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/RMA form when mailing the rotor and/or accessories.

Customers located outside the United States should contact their local Beckman Coulter office.

## **Supply List**

**NOTE** Publications referenced in this manual can be obtained at www.beckman.com or 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) or see the Beckman Coulter *Ultracentrifuge Rotors, Tubes & Accessories* catalog (BR-8101, available at www.beckman.com) for detailed information on ordering parts and supplies. For your convenience, a partial list is given below.

#### **Replacement Rotor Parts**

Description	Part Number
TLS-55 rotor assembly	346936
Buckets (set of 4)	346364
Bucket cap assembly	345770
Bucket O-ring (1)	868638
Rotor stand	345773
Bucket stand	347358
Cap and plunger assembly	349338

#### Other

**NOTE** For MSDS information, go to the Beckman Coulter website at www.beckman.com.

Description	Part Number
Tubes and accessories	see Table 1
Tube rack	348305
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

Description	Part Number
Tube Topper, cordless (Japan, US, Canada)	358312
Tube Topper rack (11-mm dia. tubes)	349387
Floating spacer removal tool	338765
Tube removal tool (Quick-Seal tubes)	361668
Fraction Recovery System (for TL-series tubes)	347828
CentriTube Slicer	347960
CentriTube Slicer blades (pkg of 10)	348299
Spinkote lubricant (2 oz)	306812
Silicone vacuum grease (1 oz)	335148
Rotor Cleaning Kit	339558
Beckman Solution 555 (1 qt)	339555
Rotor cleaning brush	339379

# Beckman Coulter, Inc. Ultracentrifuge Rotor Warranty

All Beckman Coulter ultracentrifuge Fixed Angle, Vertical Tube, Near Vertical Tube, Swinging Bucket, and Airfuge rotors are warranted against defects in materials or workmanship for the time periods indicated below, subject to the Warranty Conditions stated below.

Preparative Ultracentrifuge Rotors	5 years — No Proration
Analytical Ultracentrifuge Rotors	5 years — No Proration
ML and TL Series Ultracentrifuge Rotors	5 years — No Proration
Airfuge Ultracentrifuge Rotors	1 year — No Proration

For Zonal, Continuous Flow, Component Test, and Rock Core Ultracentrifuge Rotors, see separate warranty.

#### Warranty Conditions (as applicable)

- 1. This warranty is valid for the time periods indicated above from the date of shipment to the original Buyer by Beckman Coulter or an authorized Beckman Coulter representative.
- **2.** Maintain one copy of this software for backup purposes (the backup copy shall be supplied by Beckman Coulter);
- **3.** This warranty covers the Beckman Coulter Centrifuge Systems only (including but not limited to the centrifuge, rotor, and accessories) and Beckman Coulter shall not be liable for damage to or loss of the user's sample, non-Beckman Coulter tubes, adapters, or other rotor contents.
- **4.** This warranty is void if the Beckman Coulter Centrifuge System is determined by Beckman Coulter to have been operated or maintained in a manner contrary to the instructions in the operator's manual(s) for the Beckman Coulter Centrifuge System components in use. This includes but is not limited to operator misuse, abuse, or negligence regarding indicated maintenance procedures, centrifuge and rotor classification requirements, proper speed reduction for the high density of certain fluids, tubes, and tube caps, speed reduction for precipitating gradient materials, and speed reduction for high-temperature operation.
- **5.** Rotor bucket sets purchased concurrently with or subsequent to the purchase of a Swinging Bucket Rotor are warranted only for a term co-extensive with that of the rotor for which the bucket sets are purchased.
- **6.** This warranty does not cover the failure of a Beckman Coulter rotor in a centrifuge not of Beckman Coulter manufacture, or if the rotor is used in a Beckman Coulter centrifuge that has been modified without the written permission of Beckman Coulter, or is used with carriers, buckets, belts, or other devices not of Beckman Coulter manufacture.
- 7. Rotor parts subject to wear, including but not limited to rotor O-rings, VTi, NVT, TLV, MLN, and TLN rotor tube cavity plugs and gaskets, tubing, tools, optical overspeed disks, bearings, seals, and lubrication are excluded from this warranty and should be frequently inspected and replaced if they become worn or damaged.
- 8. Keeping a rotor log is not mandatory, but may be desirable for maintenance of good laboratory practices.

#### **Repair and Replacement Policies**

- 1. If a Beckman Coulter rotor is determined by Beckman Coulter to be defective, Beckman Coulter will repair or replace it, subject to the Warranty Conditions. A replacement rotor will be warranted for the time remaining on the original rotor's warranty.
- 2. If a Beckman Coulter centrifuge is damaged due to a failure of a rotor covered by this warranty, Beckman Coulter will supply free of charge (i) all centrifuge parts required for repair (except the drive unit, which will be replaced at the then current price less a credit determined by the total number of revolutions or years completed, provided that such a unit was manufactured or rebuilt by Beckman Coulter), and (ii) if the centrifuge is currently covered by a Beckman Coulter warranty or

Full Service Agreement, all labor necessary for repair of the centrifuge.

- **3.** If a Beckman Coulter rotor covered by this warranty is damaged due to a malfunction of a Beckman Coulter ultracentrifuge covered by an Ultracentrifuge System Service Agreement, Beckman Coulter will repair or replace the rotor free of charge.
- **4.** If a Beckman Coulter rotor covered by this warranty is damaged due to a failure of a Beckman Coulter tube, bottle, tube cap, spacer, or adapter, covered under the Conditions of this Warranty, Beckman Coulter will repair or replace the rotor and repair the instrument as per the conditions in policy point (2) above, and the replacement policy.
- **5.** Damage to a Beckman Coulter rotor or instrument due to the failure or malfunction of a non-Beckman Coulter tube, bottle, tube cap, spacer, or adapter is not covered under this warranty, although Beckman Coulter will assist in seeking compensation under the manufacturer's warranty.

#### Beckman Coulter will assist in seeking compensation under the manufacturer's warranty.

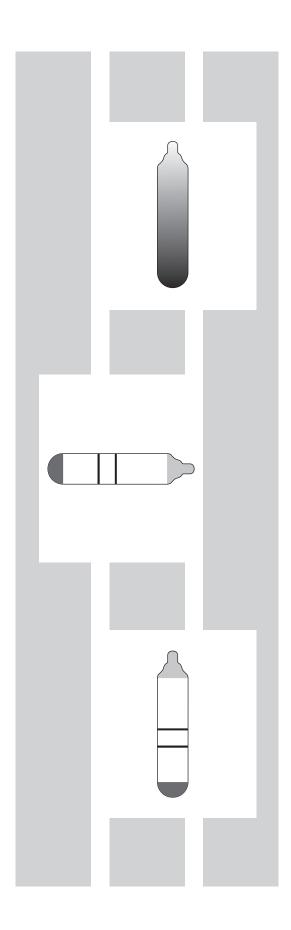
#### Disclaimer

IT IS EXPRESSLY AGREED THAT THE ABOVE WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND OF THE WARRANTY OF MERCHANTABILITY AND BECKMAN COULTER, INC. 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.

#### **Factory Rotor Inspection Service**

Beckman Coulter, Inc., will provide free mechanical and metallurgical inspection in Indianapolis, Indiana, USA, of any Beckman Coulter rotor at the request of the user. (Shipping charges to Beckman Coulter are the responsibility of the user.) Rotors will be inspected in the user's laboratory if the centrifuge in which they are used is covered by an appropriate Beckman Coulter Service Agreement. Contact your local Beckman Coulter office for details of service coverage or cost.

Before shipping, contact the nearest Beckman Coulter Sales and Service office and request a Returned Goods Authorization (RGA) form and packaging instructions. Please include the complete rotor assembly, with buckets, lid, handle, tube cavity caps, etc. A SIGNED STATEMENT THAT THE ROTOR AND ACCESSORIES ARE NON-RADIOACTIVE, NON-PATHOGENIC, NON-TOXIC, AND OTHERWISE SAFE TO SHIP AND HANDLE IS REQUIRED.



# **Related Documents**

#### Rotors and Tubes for Beckman Coulter Tabletop Preparative Ultracentrifuges (TLR-IM-9)

- Rotors
- Tubes and Accessories
- Using Tubes and Accessories
- Using Rotors
- Care and Maintenance
- Chemical Resistances
- The Use of Cesium Chloride Curves
- Gradient Materials
- References

#### Additional References

- Chemical Resistances for Beckman Coulter Centrifugation Products (IN-175)
- Ultracentrifuge Rotors, Tubes, and Accessories catalog (BR-8101)
- Use and Care of Tubes and Bottles (IN-192)

Available in electronic pdf or in hard copy by request.

Available at www.beckman.com.

www.beckman.com

