Thermo Scientific

# TZ-32 Zonal Rotor

for the Thermo Scientific WX+ Centrifuge

## Instruction Manual

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# 1. Preface

Zonal centrifugation has drawn attention in the biological, medical, and agricultural fields. Swinging bucket rotors have mainly been used for separating cellular substances, virus, and other fine particles. The advantages of the zonal rotor over the swinging bucket rotors exist in reduction of convective disturbance due to “wall effect”, large capacity, and capability of dynamic continuous loading, unloading and analytical operation of samples while the rotor is spinning. Because of the high performance and reliability, the TZ-32 Zonal rotor can be used in many fields of laboratory work and commercial production.

## 1. 1. Intended Use

The rotor is used in combination with the according centrifuge as a laboratory product designed to separate components by generation of Relative Centrifugal Force (RCF).

If these rotors are used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

These rotors should be operated by trained specialists only.

# 1. 2. Items Supplied

## 1. 2. 1. Rotor Assembly

### Rotor Components

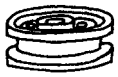
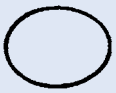
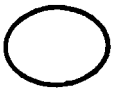
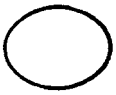
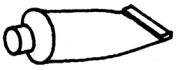
Figure 1 Rotor Components


| | |
|---|---|
| ① | Rotor cover |
| ② | Rulon seal (439345) |
| ③ | Metal ring (451152) |
| ④ | Seal packing (451153) |
| ⑤ | Cap assembly (327251A) |
| ⑥ | Stopper (455897) |
| ⑦ | Shaft (209280) |
| ⑧ | Septa (103547) |

**CAUTION** The TZ-32 zonal rotor assembly does not include the RPZ-S seal attachments assembly. It has to be ordered seperately. See “Figure 2” on page 7.

## Rotor Accessories

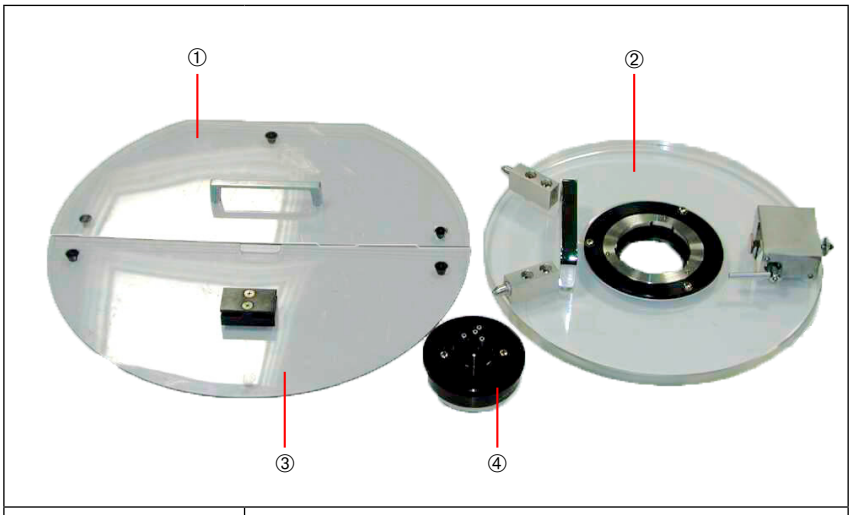
Table 1
Rotor Accessories

| Item | Article No. | Note | Quantity | Picture |
|---|---|---|---|---|
| Rulon seal | 439345 | | 3 |  |
| Rotor stand | 216715 | | 1 |  |
| Seal packing | 451153 | TZ-32 | 1 |  |
| O-ring | 8062222 | 1AS22 (JIS-B-2401)<br>For rotor | 2 |  |
| O-ring | 8062014 | 1AP14 (JIS-B-2401)<br>For Rulon seal | 2 |  |
| O-ring | 8062003 | 1AP3 (JIS-B-2401)<br>For Rulon seal | 2 |  |
| O-ring | 8062018 | 1AP18 (JIS-B-2401)<br>For shaft | 6 |  |
| O-ring | 8062218 | 1AS18 (JIS-B-2401)<br>For shaft | 2 |  |
| Stopper | 455897 | | 2 |  |
| Instruction manual | 50146926 | | 1 |  |
| Over speed decal | 34644414 | | 2 |  |
| Screwdriver | 464912 | | 1 |  |
| Tool box | 320717 | | 1 |  |
| Circlip remover | 84850103 | | 1 |  |
| Lubricant for screws | 84810201 | | 1 |  |

## 1. 2. 2. Seal Attachment Assembly

### Seal Attachment Components

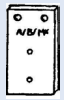
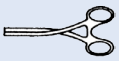
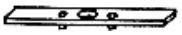
Figure 2
Seal attachment components (Type RPZ-S. Part cord. 901306)




| | |
|---|---|
| ① | Part 1 of the shield plate assembly (322897B) |
| ② | Guard plate assembly (104230A) |
| ③ | Part 2 of the shield plate assembly (323896B) |
| ④ | Seal assembly (217428A) |

## Seal Attachment Accessories

Table 2
Seal Attachment Accessories

| Item | Article No. | Note | Quantity | Picture |
|---|---|---|---|---|
| Hexagon socket head bolt | 70017308 | M6 x 30 | 6 |  |
| D6 spring washer | 80112078 | D6 | 6 |  |
| D6 flat washer | 80202088 | D6 | 6 |  |
| Tee connector | 484286 | | 4 |  |
| Rubber tube clamp | 466544 | | 6 |  |
| Tygon tube | 662905 | R-3603<br>1/8” x 1/4” x 5 m | 1 |  |
| Ball bearing | 60000178 | | 1 |  |
| Support | S408766 | | 3 |  |
| Spacer | S408765 | | 3 |  |
| Extractor | 479965 | For Rulon seal | 1 |  |
| Seal rubber | S202661 | | 1 |  |
| Snap ring remover | 84850109 | | 1 |  |
| Hexagon bar wrench | 60000122 | For M4 setscrew | 1 |  |
| Holder base | 343920A | | 1 |  |
| Handle | 323663A | | 1 |  |

## 1. 3. Precautions

- Do not spin a rotor beyond the allowable speed.
  - » The maximum speed marked on a rotor refers to an allowable one when the maximum density of the sample or density gradient solution is less than 1.2.
  - » When a sample or density gradient solution of more than 1.2 in maximum density is used, the maximum speed must be reduced according to the instruction manual for the rotor.
  - » Upon reaching the end of the service life of a rotor specified in its instruction manual, the allowable speed should be reduced by 10%.
- Set a rotor gently and securely onto the drive shaft hub.
  - » Practice setting repeatedly to acquire the knack.
- Confirm that the rotor is free from corrosion and scores.
  - » If white corrosion is recognized on a tube hole bottom of an angle rotor, or a hole bottom of a bucket for swing rotor, immediately stop using it and contact the nearest agent of Thermo Fisher Scientific
  - » When operation is over, store the rotor after cleaning and drying.
- Make it a practice to keep a logbook for rotors.
  - » Keeping a logbook for rotors is indispensable for controlling the life or when the warranty clause is involved. Keep the logbook up to date and store it properly.
- Do not rotate this rotor at a speed of more than 5000 rpm unless all components (rotor assembly, cap assembly) are assembled.

  Do not rotate this rotor at a speed of more than 5000 rpm unless the rotor is filled with liquid.

  If the above cautions are not observed, septa could be damaged.
- Always ground the ultracentrifuge before use.

## 1. 4. Symbols used in the manual

This symbol refers to general hazards.
CAUTION means that material damage could occur.
WARNING means that injuries or material damage or contamination could occur.

This symbol refers to biological hazards.
Obey the safety information contained in the instruction manual to keep you and your environment safe.

# 2. Technical Specifications

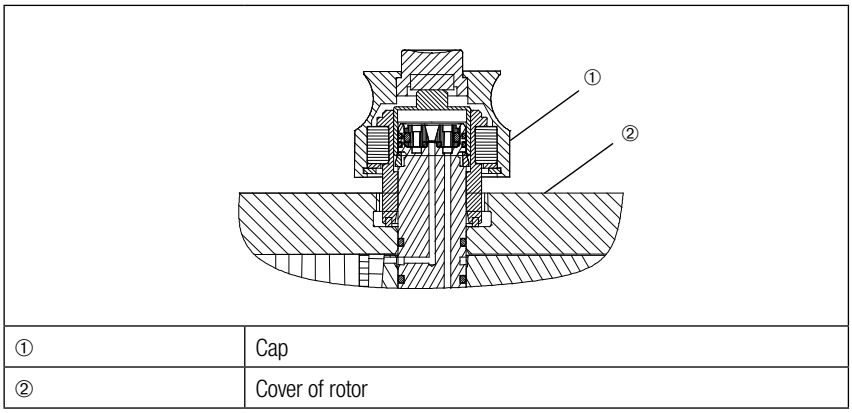
**Table 3** Technical Specifications

| | |
|---|---|
| Maximum Speed | 32000 rpm |
| Maximum Centrifugal Force | 102000 x g |
| K-factor | 363 |
| Capacity | 1690 ml |
| Maximum Density of Gradient Solution | 1.2 g/ml |
| Maximum Radius | 8.89 cm |
| Rotor Material | Titanium Alloy |
| Weight | 15.4 kg |

# 3. Construction

## 3. 1. High-Speed Rotation

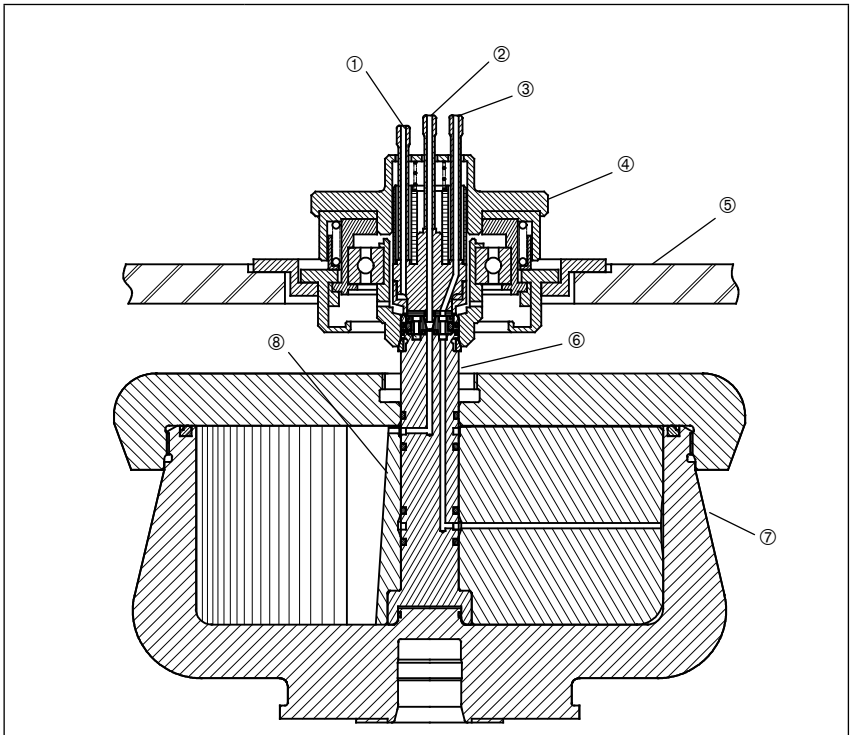
Figure 3
Construction – High Speed Rotation




| | |
|---|---|
| ① | Cap |
| ② | Cover of rotor |

## 3. 2. Loading and Unloading

Figure 4
Construction – Loading and Unloading




| | |
|---|---|
| ① | Pipe for cooling water |
| ② | Pipe in the rotor center |
| ③ | Pipe on the outer wall of rotor |
| ④ | Seal assembly |
| ⑤ | Guard plate |
| ⑥ | Shaft |
| ⑦ | Rotor |
| ⑧ | Over-speed decal |
| ⑨ | Septa |

# 4. Assembly

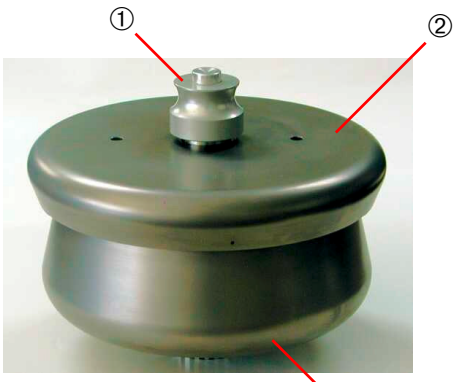
## 4. 1. Rotor Assembly

The rotor is composed of a rotor body, cover, septa, shaft, cap assembly and Rulon™ seal attached to the shaft. The TZ-32 rotor has a capacity of 1690 ml.

### 4. 1. 3. Rotor body and cover

The rotor body and cover of the TZ-32 rotor use, as material, titanium alloy of an excellent corrosion resistance. The seal packing is fluorine rubber. The metal ring is titanium alloy.

**Figure 5**
Rotor body and cover




| | |
|---|---|
| ① | Cap assembly |
| ② | Cover |
| ③ | Rotor body |

### 4. 1. 4. Septa

The septa has four vanes that divide the bowl into four sector-shaped compartments, each vane containing a channel to carry fluid to the outer edge of the rotor bowl. The solution injected through the seal assembly is led to the outer edge of the rotor from the four holes located outside the Rulon seal through the shaft. The septa has slanting surfaces, the top of each being provided with a hole communicating from the center of the seal assembly with the hole in the center of the Rulon seal. Otherwise, the septa is made of a plastic (Noryl) for which the chemical resistance, cautions in spinning, and the maintenance will be described later.

**Figure 6**
Septa

### 4. 1. 5. Shaft

A shaft is inserted in the central part of septa, and form a sample passage way. And, the tops of the shaft become the installation of the cap and the connection part of seal assembly. The material of the shaft uses titanium alloy.

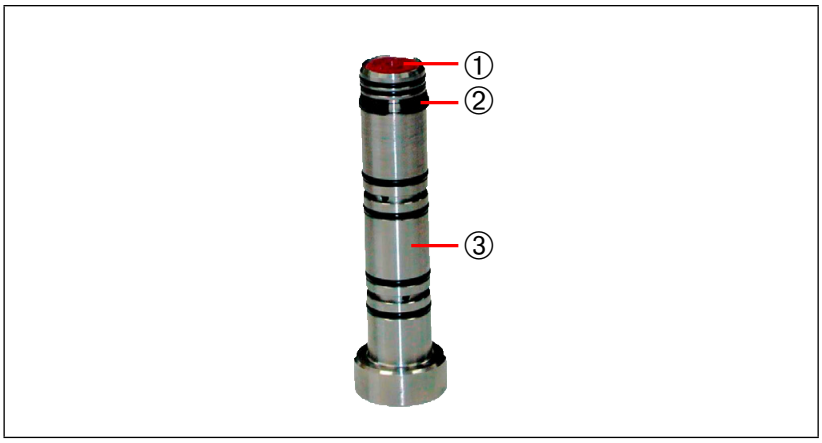
### 4. 1. 6. Stopper

A stopper is the coming off stop part when a cap is installed, and material is Noryl.

### 4. 1. 7. Rulon seal

The Rulon seal mouted on the top of shaft. This part is important component in loading and unloading, and material is fluorine plastic.

Figure 7
Shaft, Stopper and Rulon Seal




### 4. 1. 8. Cap assembly

The cap is mounted onto the shaft with the seal assembly removed after injecting a sample into the rotor. It seals the sample in the rotor when evacuating the rotor chamber and spinning the rotor at a high speed.

The cap can be easily and securely removed by pushing the extruded portion (push button) atop the cap.

Figure 8
Cap assembly

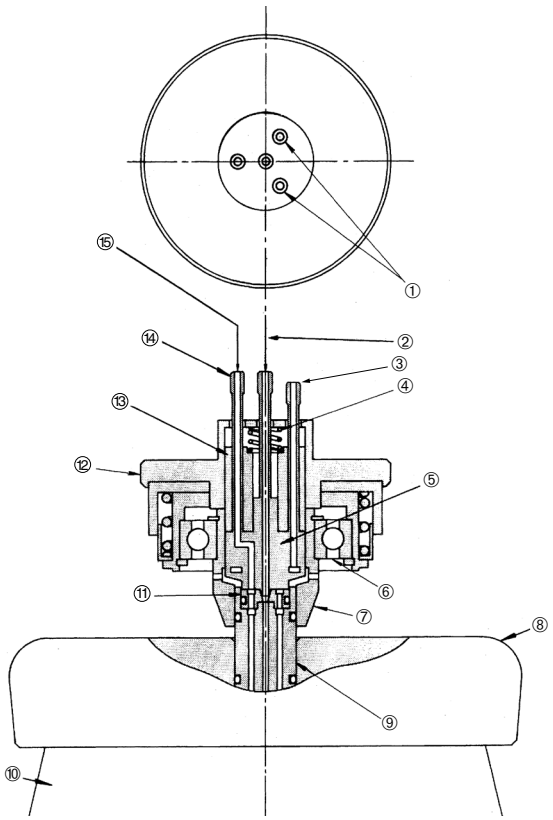
# 4. 2. RPZ-S Seal Attachment Assembly

## 4. 2. 9. Seal assembly

The seal assembly is mounted onto the rotor shaft as shown in Figure 10. The rotor, shaft, and adapter rotate, but the other components do not rotate. The outer side of the holder cover is supported by the guard plate which will be described later. The solution injected through the pipe is introduced through the body and Rulon seal into the rotor being spinned. One of the two higher pipes projecting from the body, which is located on the outer wall side of the rotor, is led to the outer groove of the Rulon seal and the outer wall of the rotor from the four septa vanes through the four holes of the Rulon seal. The pipe in the rotor center is led to the four holes on top of the septa through the center of the shaft. The two lower pipes are used for circulating cooling water into the interior of the body.

Figure 9
Seal assembly

**Figure 10**
Construction of the seal assembly

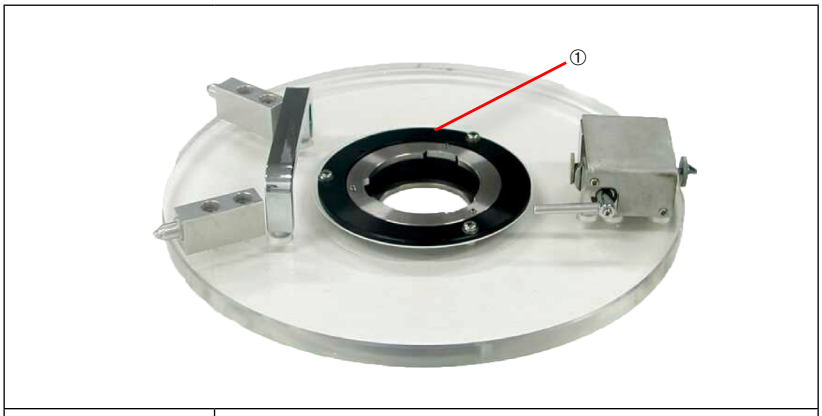



| | |
|---|---|
| ① | Body cooling water pipes |
| ② | Pipe in the rotor center |
| ③ | Cooling water pipe |
| ④ | Coil spring (B) |
| ⑤ | Body |
| ⑥ | Seal Assembly |
| ⑦ | Ball bearing |
| ⑧ | Adaptor |
| ⑨ | Cover |
| ⑩ | Shaft |
| ⑪ | Rotor body |
| ⑪ | Rulon seal |
| ⑫ | Holder cover |
| ⑬ | Guide body |
| ⑭ | Pipe |
| ⑮ | Pipe on the outer wall side of rotor |

## 4. 2. 10. Guard plate assembly

The center case supports the seal assembly and maintains the contact between the body and the Rulon seal for injecting solution into and extracting it, from the spinning rotor. It is mounted by using the three supports leading from the rotor chamber of the centrifuge. It is made of a transparent acryl resin plate for the spinning rotor to be visible. It also prevents tools, etc. from dropping into the rotor chamber while it is spinning.

**Figure 11**
Guard plate assembly




| | |
|---|---|
| ① | Case |

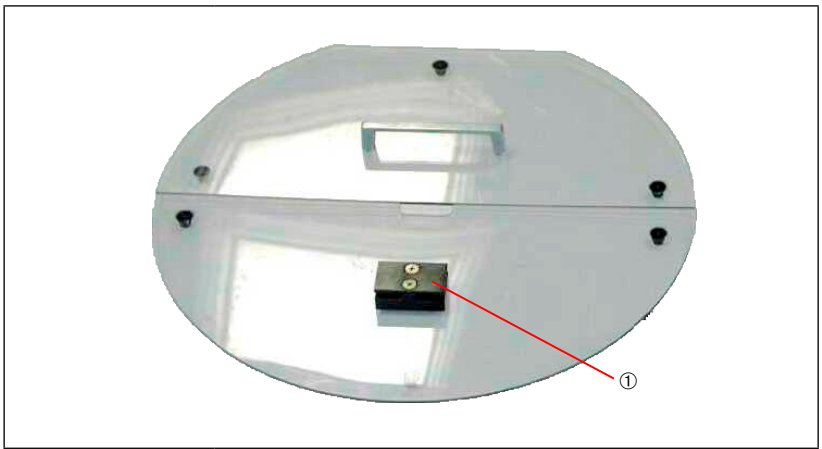
## 4. 2. 11. Shield plate

It is mounted on top of the rotor chamber to suppress frosting in the chamber when injecting gradient solution or samples into or extracting them from the rotor which is being cooled.

Since the shield plate is also made of a transparent acrylic resin plate, the rotor chamber is clearly visible through this plate.

A tube setter is provided for retaining the tubes projecting from the seal assembly. Since the shield plate is divided into two semicircular portions, it is easily mountable or removable.

**Figure 12**
Shield plate




| | |
|---|---|
| ① | Tube setter |

# 5. Operation

| ⚠ | CAUTION |
|---|---|
| Never spin the rotor at a speed over 5,000 rpm unless it is filled with the liquid.<br>If this is neglected, the septa will be damaged. | |

| ⚠ | CAUTION |
|---|---|
| The over-speed decal, if corroded or discolored must be replaced immediately.<br>The over speed decal is important to control the rotor revolution speed.<br>The over-speed decal must match the maximum speed of the rotor.<br>When replacing be very careful not to damage the over-speed decal and rotor body. | |

## Replacing over-speed decal

To replace the over-speed decal follow this procedure:

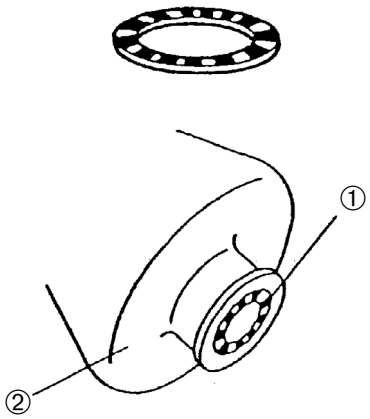
1. Preparation
   - » Prepare a new over-speed decal and knife.
   - » Wash and then dry the rotor well.
2. Hold the rotor upside down.
3. Pry the edge of the over-speed decal with the knife and remove the decal.

   Be very careful not to damage the rotor.
4. Clean the decal hole on the rotor by using alcohol.
5. Remove the backing paper from the new over-speed decal.

   Place the over-speed decal so that it fits into the groove of the decal hole on the rotor.

   Make sure the decal does not move.

Figure 13
Overspeed decal
View from the bottom




| Part No. 34644414 | |
|---|---|
| ① | Over-speed decal |
| ② | Rotor |

## Maximum Speed

The maximum speed of the rotor inscribed on the rotor cover surface is determined using the maximum density of gradient solution below 1.2 g/ml.

If the maximum denstity of gradient solution exceeds 1.2 g/ml, decrease the maximum speed of the rotor according to the following equation:

$$Allowable\ maximum\ speed = Maximum\ speed\ \boldsymbol{x}\ \sqrt{\frac{1.2}{Maximum\ density\ of\ gradient\ solution\ (g/ml)}}$$

## Employed Materials

The following materials are used on the passage of the sample:

Table 4
Employed materials

| Part name | Material |
|---|---|
| Tygon tube | PVC (specially processed) |
| Tee | Polypropylene |
| Metallic section of seal attachment assembly etc. | Stainless steel |
| Stationary seal surface (seal assembly side) | Tungsten carbide |
| Rulon seal | Tetrafluorethylene resin (Rulon) |
| O-Ring | NBR |
| Rotor, cover, shaft, metal ring | Titanium alloy |
| Septa | Noryl (polyphenylene oxide resin) |
| Seal packing | Fluorine rubber |

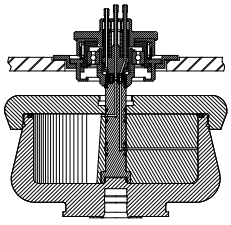
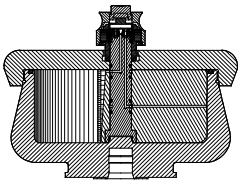
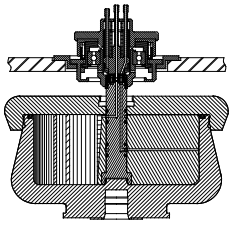
Table 5
Chemical
Resistance of Noryl

| CAUTION<br>Do not use the following substances with Noryl | | |
|---|---|---|
| Benzene | Fluorine | Petroleum ether |
| Carbon tetrachloride | Freon | Polypropylene glycol |
| Chlorine | Furfural | Pyridine |
| Chloroform | Heptane | Pyridine sulfate |
| Dibutyl phthalate | Hexanol | Toluene (toluol) |
| Ethylene glycol | Kerosene | |
| **The following substances may be used with Noryl at 26 °C or below** | | |
| Acetone | Copper sulfate | Mineral oil |
| Acetic acid | Cyclohexane | Phenol |
| Arsenic acid | Ethyl acetate | Potassium bichromate |
| Alcohol | Ethyl chloride | Potassium carbonate |
| Alkalies | Ferric chloride | Potassium chlorate |
| Ammonium chloride | Formic acid | Potassium chloride |
| Ammonium hydroxide | Hydrobromic acid | Potassium hydroxide |
| Ammonium phosphate | Hydrochloric acid | Potassium nitrate |
| Ammonium sulfate | Lactic acid | Potassium sulfate |
| Amyl acetate | Nitric acid | Sodium bicarbonate |
| Amyl alcohol | Ortho-phosphoric acid | Sodium bisulfate |
| Borax | Picric acid | Sodium carbonate |
| Bromine | Phosphoric acid | Sodium chlorate |
| Butyl alcohol | Stearic acid | Sodium cyanide |
| Butyl cellosolve | Sulfuric acid | Sodium hydroxide |
| Butyl mercaptan | Ferrous sulfate | Sodium nitrate |
| Benzoic acid | Formaldehyde | Sodium perborate |
| Boric acid | Glycerin, glycerol | Sodium peroxide |
| Butyric acid | Hydrogen peroxide | Sodium phosphate |
| Calcium chloride | Iodine | Sodium pyrophosphate |
| Calcium hydroxide | Isopropanol | Sodium silicate |
| Carbon bisulfide | Lead acetate | Sodium sulfate |
| Cesium chloride | Lithium chloride | Sodium sulfite |
| Chloracetic acid | Magnesium chloride | Sodium thiosulfate |
| Chlorosulfonic acid | Magnesium hydroxide | Stannous chloride |
| Citric acid | Methanol | Sucrose |
| Copper chloride | Methyl cyclopentane | Tetranitromethane |
| Copper nitrate | | |

# 5. 1. General Description

The zonal rotor is employed in the following manner.

Table 6
Separation Procedure

| Item \ Procedure | Loading | Centrifugal Separation | Unloading |
|---|---|---|---|
| **Solution flow** |  |  |  |
| **Speed** | 3,000 rpm | Specified speed | 3,000 rpm |
| **Procedure** | Inject density gradient, sample, overlay solution | Separation | Inject high density extrusion and recover separated solution |

Mount the seal assembly on the rotor being turned at a speed of 3000 rpm, and load a density gradient solution, a sample and overlay solution under the atmospheric pressure with the door of the centrifuge open. Then, remove the seal assembly and place the cap assembly. After closing the door of the centrifuge, start the rotor and raise the rotor speed up to a predetermined speed.

After the centrifugation reduce the rotor speed to 3000 rpm remove the cap assembly, mount the seal assembly again and unload the solution from the rotor by feeding heavy solution into the edge of the rotor. The gradient solution containing sedimented particles is unloaded by feeding a higher-density solution at the edge of the rotor. Also, the absorbancy of the solution thus unloaded is fractioned by a fraction collector while it is being measured by a spectrophotometer. In a common use, prepare the density gradient in the order of high to low density layers from the center to the edge of the rotor. Feed as “cushion” to the gradient solution of higher density a solution of a density equivalent to or higher than the maximum density of the solution. This prevents the heaviest particles from reaching the edge of the rotor when a sample is centrifuged.

After loading the “cushion” stop the pump. Load a sample to be centrifuged beginning with the center of the rotor after road the gradient solution and also load “overlay” or a lower - density solution, so that the sample is layered cylindrically in the rotor. (The sample is loaded at the edge of the rotor as the case may be.) Then, a portion of the cushion previously loaded is discharged in an amount equivalent to the amount of the sample and overlay thus loaded.

# 5. 2. Preparation

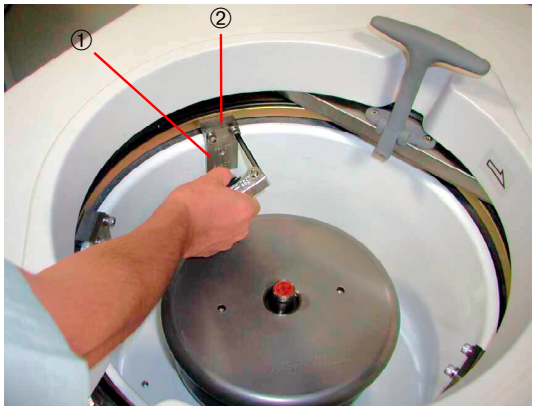
## 5. 2. 1. Prepare the Centrifuge

### Mount the supports

a. Mount the three supports onto the fixed pieces in the rotor chamber of the centrifuge as shown in Fig.5-1.

b. Center and adjust height of guard plate.

Figure 14
Mounting the supports

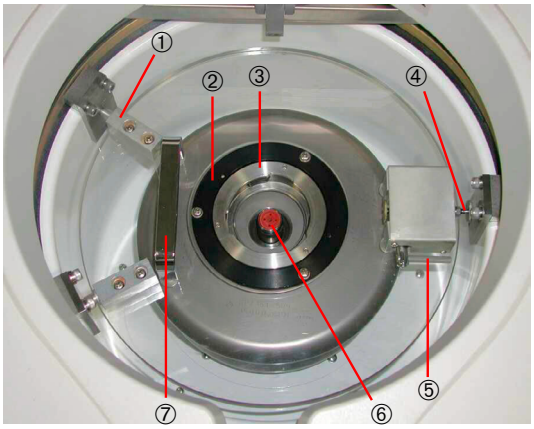



| | |
|---|---|
| ① | Support |
| ② | Fixed piece |

## Mounting the guard plate

Put the rotor on the spinning shaft and mount the guard plate.(In TZ-32, mount it to the upper hole in the support.) The guard plate can be fixed by inserting first the end of the fixed block into the left hole on the support, erecting the right knob and then turning it to horizontal.

Figure 15
Centering the guard plate

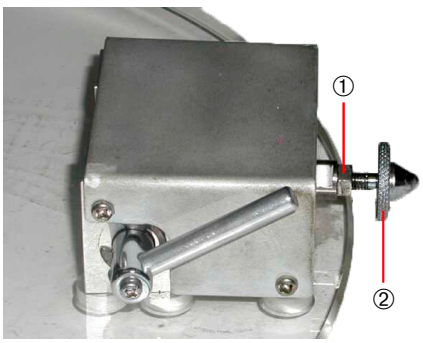



| | |
|---|---|
| ① | Fixed block |
| ② | Base |
| ③ | Case |
| ④ | Settler |
| ⑤ | Knob |
| ⑥ | Shaft |
| ⑦ | Handle |

## Adjusting the base

a. Loosen the four upper screws in the fixed block to make it movable. Then, loosen the lock nut of the setter. Move the fixed block and setter back and forth (by turning the setter) until the acryl plate is aligned with the rotor center. Tighten the screws to fix them.

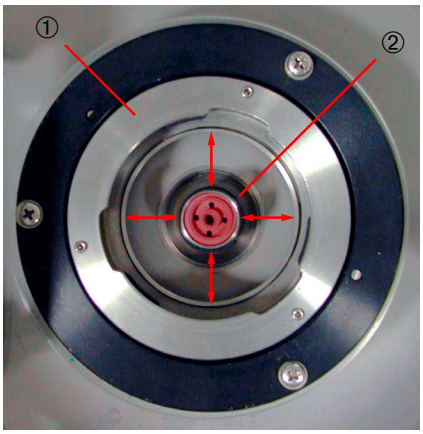
Figure 16
Loosening the lock nut




| | |
|---|---|
| ① | Lock nut |
| ② | Setter |

b. Loosen the four screws in the base, and adjust until the center of the rotor shaft coincides with that of the base. Tighten the screws and fix the base. Adjust the case, while visual checking, until it is concentrical with at the center of the shaft.

Figure 17
Adjusting the base

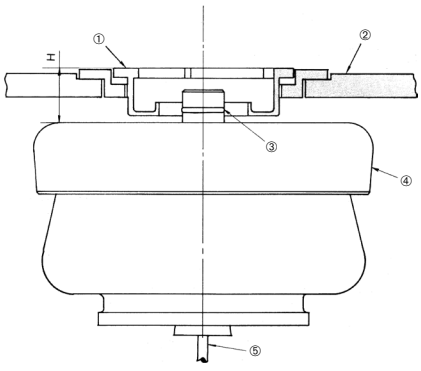



| | |
|---|---|
| ① | Case |
| ② | Shaft |

## Height adjustment of case

Adjust the height of the rotor and case as shown in Figure 18.

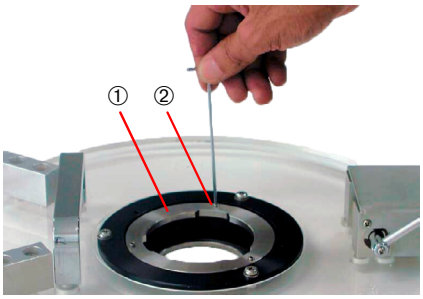
Figure 18
Height adjustment




| | |
|---|---|
| ① | Case |
| ② | Guard plate |
| ③ | Shaft |
| ④ | Rotor |
| ⑤ | Spinning Shaft |

The height of the case is adjusted as follows; Loosen the three setscrews by the hex. bar wrench accessory to this rotor, and the case can be turned. Turning clockwise lowers it, while turning counterclockwise lifts it. After adjustment, tighten the three setscrews to fix the case.

**Figure 19**
Tightening the three setscrews



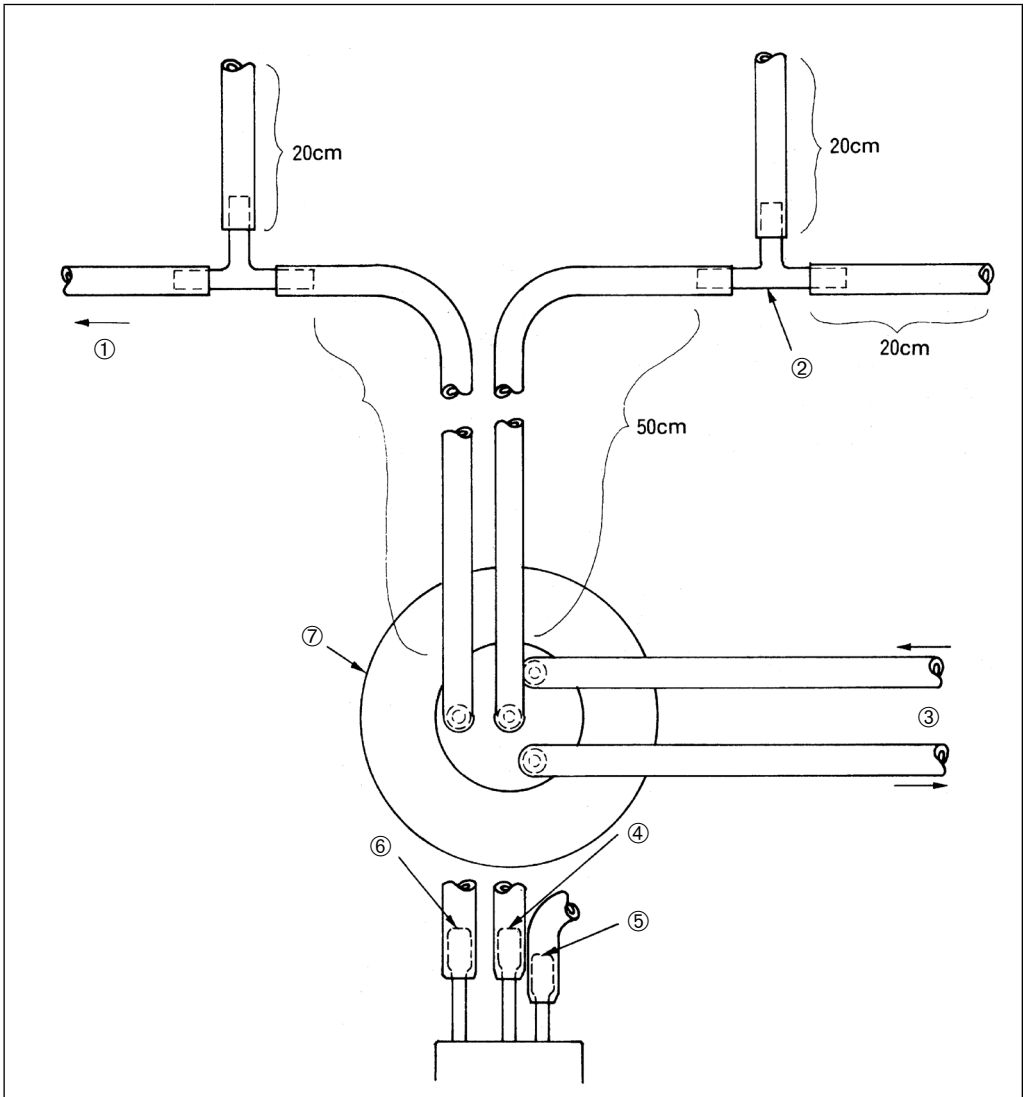

| | |
|---|---|
| ① | Case |
| ② | Guard plate |

## 5. 2. 2. Connect a tube to the seal assembly

### Tube connection

For connecting the tubes, see Figure 20. The two higher ones of the four pipes led from the seal assembly are used for injection into or extraction from the rotor. The outer one is led to the outer wall of the rotor and the center one is led to the center of the rotor. The two remaining pipes are used for cooling gradient solution and samples when necessary. Use pipes with an inner diameter of 3.2 millimeter (1/8”).

Figure 20
Tube connection




| | |
|---|---|
| ① | Pump |
| ② | Tee |
| ③ | Cooling water |
| ④ | Pipe on the rotor center |
| ⑤ | Cooling pipes (lower at the top end than the other two pipes) |
| ⑥ | Pipe on the outer wall of the rotor |
| ⑦ | Seal assembly |

### Cooling water

Cooling water is needed for cooling the contact body, which touches the Rulon seal. Use city water as coolant in case centrifuge is to be done at normal temperature. However use cooled water when centrifuge is to be done at a temperature below 5 °C. The minimum flow rate required is 450 ml/min.

## 5. 2. 3. Confirm test operation

Check the seal assembly for leakage before injecting a gradient solution into the rotor. This check should be done by injecting distilled water into the rotor by the pump or the injector. The operation and handling are the same as in injecting gradient solution.

Check the seal assembly in the following procedure.

a. Set the rotor and guard plate, and regulate the rotor speed to 3000 rpm.

b. Mount the seal assembly with the tubes connected.

c. Inject about 100 ml of distilled water by the pump or injector through the pipe leading to the outer wall of the rotor.

Leakage from the seal assembly, if any, can be detected from much splashed injected solution sticking to the rotor chamber of the centrifuge. If any leakage is detected, check as follows before performing the preliminary operation again. Refer to item 7 for the detailed check.

» Check that the Rulon seal is mounted onto the shaft normally.

(Check that the O-ring is mounted or if there is any scratch on the top surface.)

» Check that the seal assembly body operates smoothly.

(Check the contact between the Rulon seal and contact body of seal assembly, and also the spring which depresses the guide body.)

» Check if the outlet pipe on the rotor center blocks normal flow of solution.

For repair of the Rulon seal surface, see „6. 4. Handling of Rulon Seal and Renewal“ on page 44.

# 5. 3. Operation

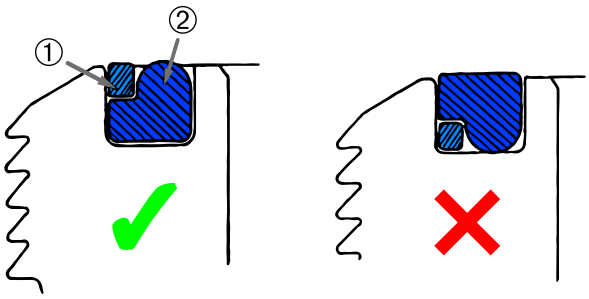
## 5. 3. 1. Prepare the Rotor

1. Put the seal packing and metal ring into the rotor. Put the O-ring into the extrusion on the center bottom of the rotor. Apply a thin coat of vacuum grease to the seal packing and O-ring.

**CAUTION** Set the seal packing and metal ring correct. If they are set incorrect, the seal packing comes out of the seal assembly, thus possibly causing the leakage of solution and damaging the rotor.

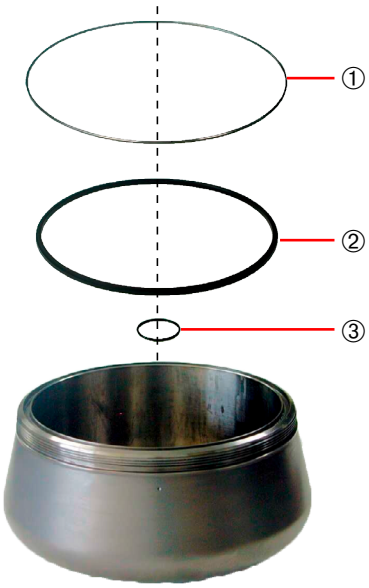
**CAUTION** Since O-ring and seal packing which are used in the rotor or the connecting portion of the seal section will come into contact with sample, always rinse them before use. When these parts are replaced, some residue generated when rubber was formed may remain on the surfaces of new parts. Rinse them away before use.

Figure 21
Setting the seal packing in detail




| | |
|---|---|
| ① | Metal ring |
| ② | Seal packing |

Figure 22
Setting the seal packing




| | |
|---|---|
| ① | Metal ring |
| ② | Seal packing |
| ③ | O-Ring (1AS22) |

2. Put the Rulon seal and O-Ring into the shaft.

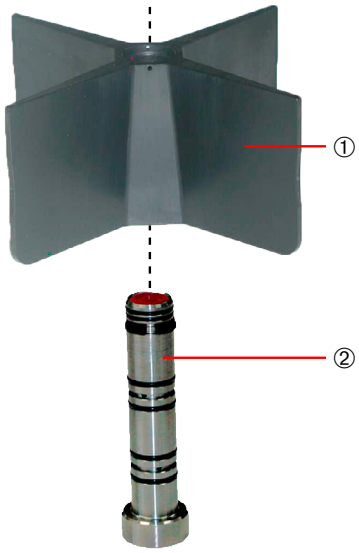
**CAUTION** Be careful not to scratch the Rulon seal surface by a fingernail. When inserting the seal, carefully push it with the soft portion of finger applied. Apply a thin coat of vacuum grease to the O-ring.

Figure 23
Assembling the Rulon seal and O-ring on the shaft


| | |
|---|---|
| ① | Rulon seal |
| ② | O-Ring (1AP14) |
| ③ | O-Ring (1AP3) |
| ④ | O-Ring (1AS18) |
| ⑤ | Stopper |
| ⑥ | O-Ring (1AP18) |
| ⑦ | Shaft |

3. Put the shaft into the septa.

**Figure 24**
Shaft into septa



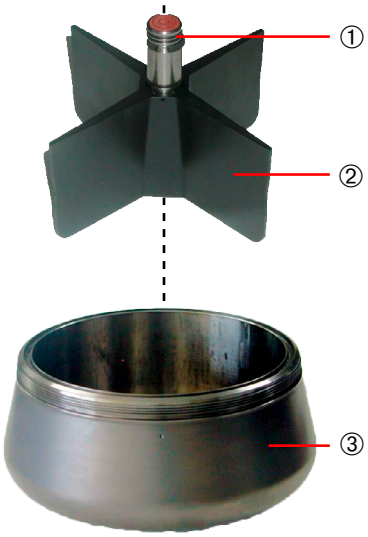

| | |
|---|---|
| ① | Septa |
| ② | Shaft |

4. Put the assembled septa into the rotor.

   Fit the hole in the bottom end of the shaft onto the extrusion on the rotor bottom where the O-ring (1AS22) is inserted.

   Thus, the upper surfaces of both rotor and septa will be flush with each other.

**Figure 25**
Assembled septa into rotor



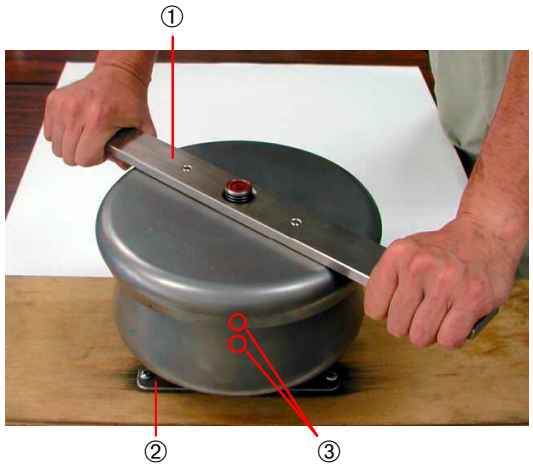

| | |
|---|---|
| ① | Shaft |
| ② | Septa |
| ③ | Rotor |

5. Put the cover onto the rotor containing the septa.

   Put the rotor on the fixed holder base and screw in the cover by hand. Mount the handle as shown in the figure to fasten the cover.

   **CAUTION** Fasten the cover until the match mark on the cover meets that on the rotor body. As the rotor is used over and over again, the match mark on the cover will go over that on, the rotor body, which, however, will result in no trouble.

Figure 26
Assemble the rotor cover




| | |
|---|---|
| ① | Handle |
| ② | Holder base |
| ③ | Match marks |

## 5. 3. 2. Preparation for spinning the rotor

1. Mount the rotor on the spinning shaft in the rotor chamber of the centrifuge.

2. Mount the guard plate.

   Secure the fixed piece on the left support first and fix it by turning over the right-hand support toward the right support.

   For setting the supports use the marked upper support hole. Neither mark A nor mark B is employed in the zonal rotor.

Figure 27
Mount the guard plate

## 5. 3. 3. Spinning

**CAUTION** Never raise the rotor speed to more than 5000 rpm with the rotor not filled with the solution to full extent (less than 90%). High speed spinning of the rotor not full of the solution will cause the septa to be damaged. The procedure of spinning the zonal rotor depends on the type of ultracentrifuge used with the rotor. For spinning the rotor carefully read and follow the instructions given below and in the instruction manual of the centrifuge.

Table 7
Operation modes

| Speed set to 3000 rpm by acceleration | High speed spinning | Speed set to 3000 rpm by deceleration |
|---|---|---|
| 1. Set to ZONAL operation mode from panel.<br>2. Set operating factors for high speed spinning.<br>3. Press the START switch. | 1. Close the door.<br>2. Press the VACUUM switch.<br>3. Press the START switch. | 1. When the timer counts down to "0", the rotor decelerates to 3000 rpm.<br>2. Press the VACUUM switch.<br>**NOTE** When it is desired to stop the rotor, set to the normal operation mode on the panel. |

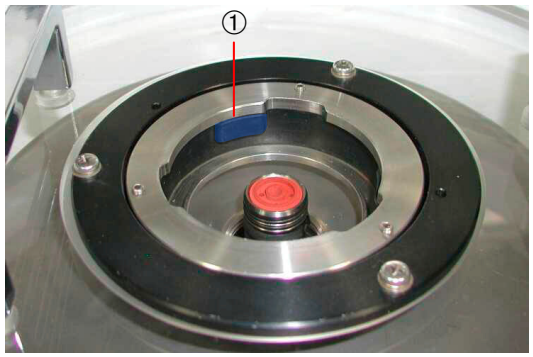
For details on operating from the panel, refer to the instruction manual of the centrifuge.

## 5. 3. 4. Injection of gradient solution and sample

1. Mount the seal assembly.

   Depress the three extrusions of the seal assembly by fitting them into the three relief grooves. Then, the guide at the end of the seal assembly enters the rotor shaft. Turn the seal assembly clockwise and release it when it touches the rubber stopper.

Figure 28
Mount the seal assembly

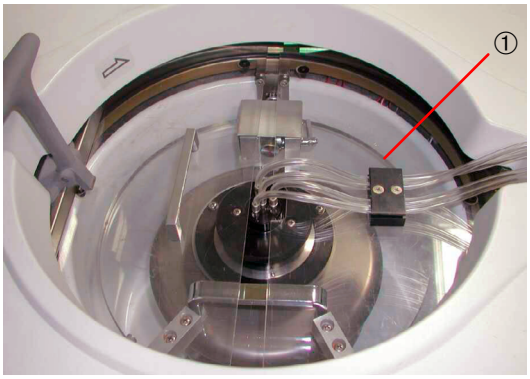



| | |
|---|---|
| ① | Rubber stopper |

2. Mount the shield plate.

   Put the tube setter side of the shield plate to right or left end of the rotor chamber in the centrifuge. Insert the tubes from the shield plate into the tube setter through the notch of the shield plate. Mount another shield plate, and position it so that there is no clearance at the center.

**Figure 29**
Mount the shield plate




| | |
|---|---|
| ① | Shield plate |

3. Feed a gradient solution by pump.

   a. Pipe on the outer wall of the rotor

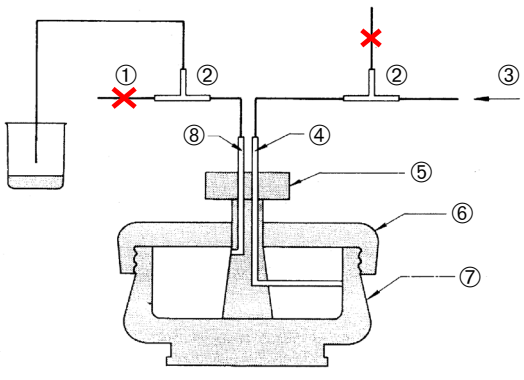
      Connect one end to the pump and the other one to the clamp.

   b. Pipe on the rotor center

      Put one end of the pipe into a beaker containing water. Make sure gradient solution is infected. Clamp the other end.

      **CAUTION** at the time of infecting gradient solution. Select such on infection speed of gradient solution that infection pressure of the pump (pipepressure at infecting side of the seal is below 1 kg/cm²). Special care should be exercised in selecting an infection speed for a high-viscosity liquid such as sucrose (For 60% solution of sucrose the infection speed is limited to approximately 35 ml/min.)

**Figure 30**
Feed a gradient solution by pump



4. Injection of sample and overlay

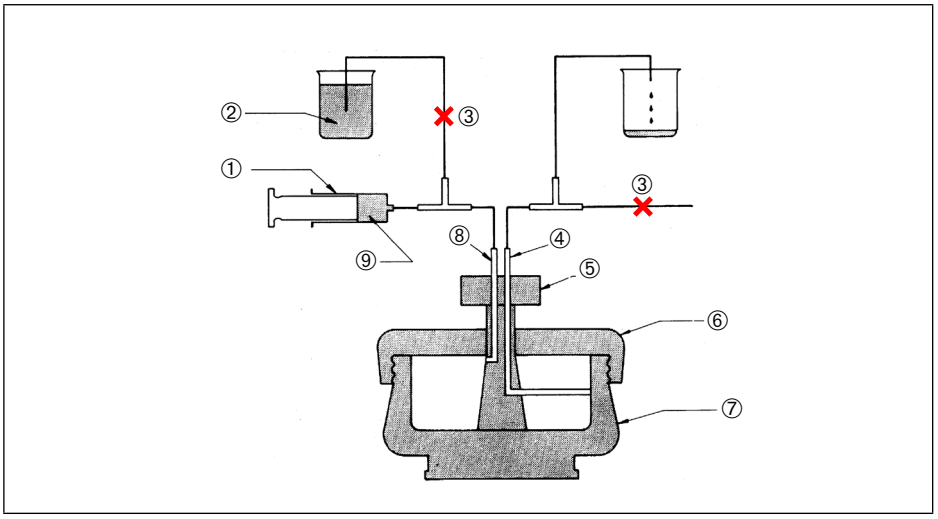
   a. Pipe on the outer wall of the rotor

      Clamp one end of the pipe at an outer position from the tee on the pump side, and put the other end into a beaker for receiving the “cushion” discharged due to injection of sample and overlay.

   b. Pipe on the rotor center

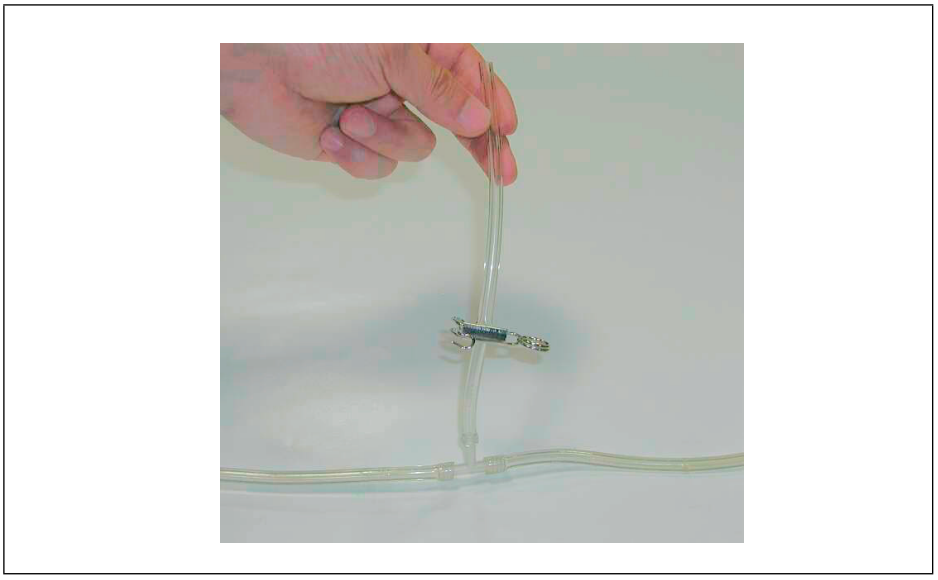
      When the lightest gradient solution has filled the tee, clamp both ends of the pipe and stop the pump.Inject a sample through one end by an injector. Then feed an overlay of 75 ml into the rotor.

**Figure 31**
Injection of sample and overlay




**CAUTION** Do not feed bubbles while injecting a density gradient solution and sample. Otherwise the gradient solution and sample will be disturbed. If bubbles are about to enter, lead them to another tube from the tee by setting upright the tube containing bubbles before they passthrough.

**Figure 32**
How to avoid bubbles while injecting a density gradient solution and sample

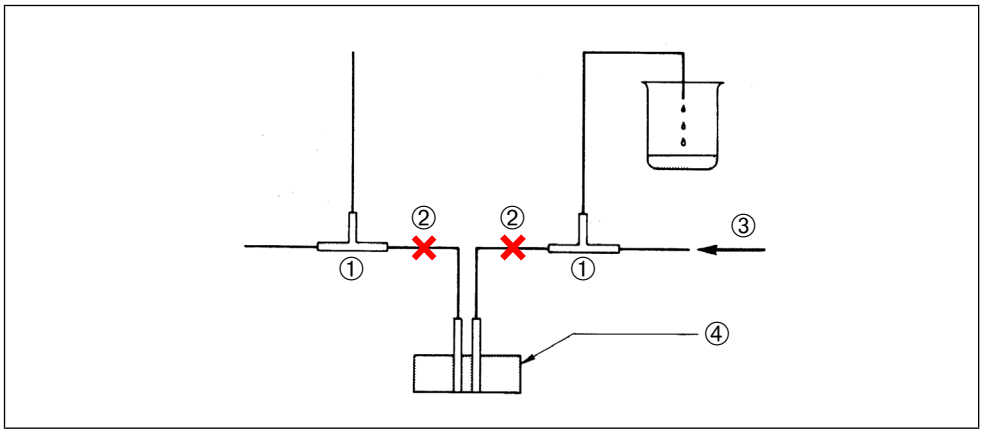


5. Preparation of the pump

   Pipe on the outer wall of the rotor and rotor center

   Clamp each pipe between the tee and the seal assembly, and remove the seal assembly. Flow a heavy extracting solution through the outerwall of rotor line of the clamped pipe and eliminate bubbles from the pipe on the outer wall of rotorline.

**Figure 33**
Preparation of the pump




6. Removal of air bubbles

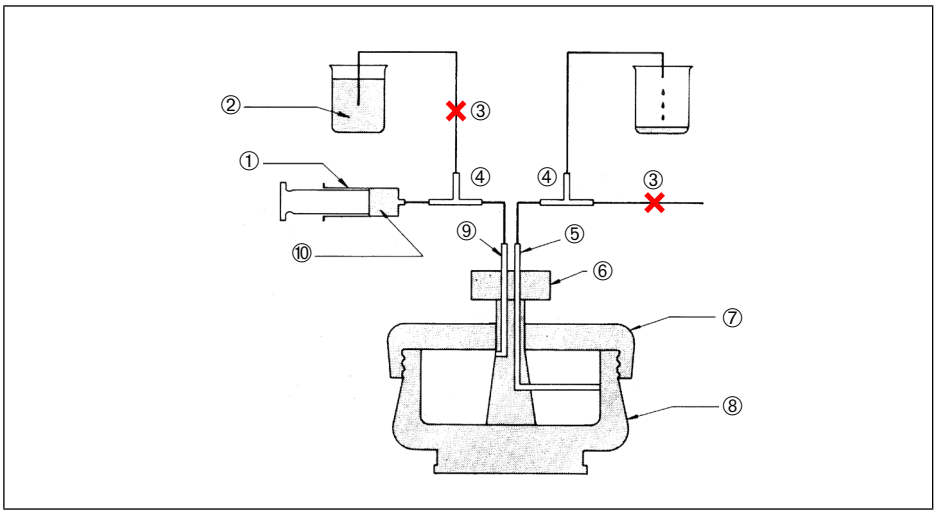
   a. Pipe on the outer wall of the rotor

      Clamp the pipe on the pump side.

   b. Pipe on the rotor center

      Prepare about 15 ml of an overlay, disconnect the clamp on the rotor side, and clamp the other end for injection. Bubbles will be removed through the pipe on the rotor center.

**Figure 34**
Removal of air bubbles




7. Remove the shield plate and remove the seal assembly.

   Remove the tubes from the tube setter and then the shield plate. The seal assembly can be pulled ipwards when it touches the stopper while turning it counterclockwise by hand.

## 5. 3. 5. High speed spinning

1. Place the cap

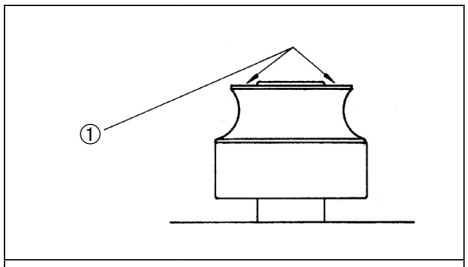
   Hold the upper fringe as shown in the figure, and push it onto the shaft downwards.

   **CAUTION** Make sure that the cap, once set completely, will not come off the shaft. Even when it is pulled holding the fringe. It is recommended to try setting the cap into the rotor as preliminary practice before spinning the rotor.

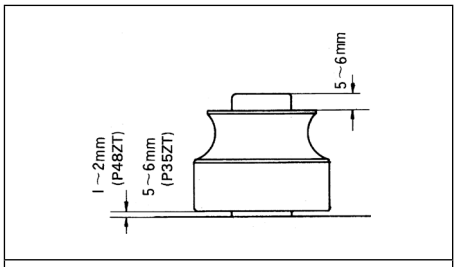
Figure 35
Place the cap

2. Referring to the illustrations, check that the cap is set completely.

Figure 36
Checking the cap




| The cap is set incompletely. | |
|---|---|
| ① | Push the fringe |




| The cap is set completely. |
|---|
| |

3. For the high speed spinning, see „5. 3. 3. Spinning“ on page 33.

## 5. 3. 6. Deceleration and extraction

1. Regulation of the rotor speed to 3000 rpm by deceleration, see „5. 3. 3. Spinning“ on page 33.
2. Push the R. PUMP button and stop the vacuum pump to let air into the rotor chamber.
3. Remove the cap.

   Hold the running cap by both hands. Pull it upwards while pushing the center extrusion.
4. Mount the seal assembly.

   See “5. 3. 4. Injection of gradient solution and sample” on page 33.
5. Unloading the separated soultion by pump

   a. Pipe on the outer wall of rotor

      Feed by pump with a heavy solution having a density equivalent to or higher than the maximum density of the

gradient solution.

Clamp other end.

a. Pipe on the rotor center

Clamp one end. Lead the other end to a fraction collector through a spectrophotometer flow cell.

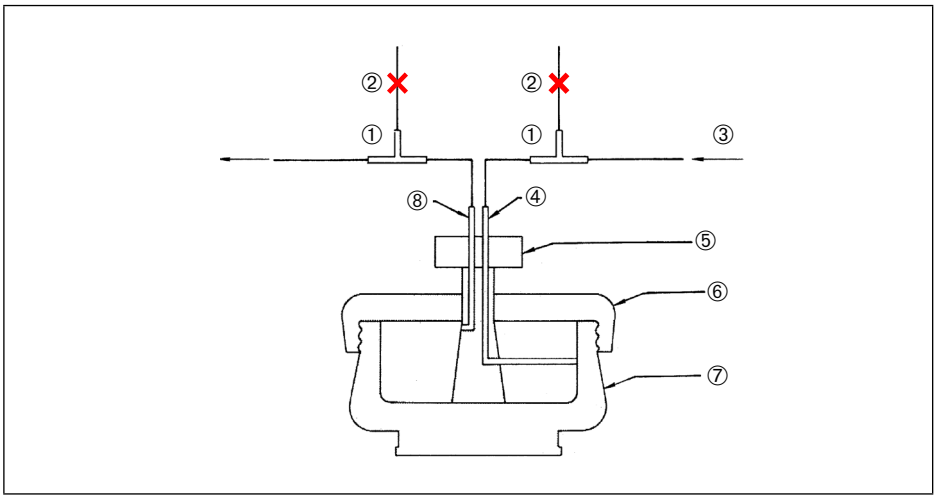



**Figure 37**
Deceleration and extraction

# 5. 4. Operational Checkpoints

## 5. 4. 1. Setting of sample temperature and rotor temperature

Make the temperature of injecting sample and density gradient solution and the setting temperature of a rotor when spinning as equal as possible. If not, following problems may occur:

1. When the temperature of injecting solution is lower than the temperature of the spinning rotor:

   Because of the temperature rise of the solution injected into the rotor, the volume expands and the pressure inside the rotor increases.The push button of the cap assembly cannot be pushed down and the cap cannot be removed. As the result, it is impossible to unload. Following table shows the quantities of increased volumes when using the TZ-32 rotor and water as sample.

Table 8
Increase of Volume

| Temperature Rise | Increase of Volume |
|---|---|
| 5 °C ➞ 15 °C, 10 K | 1.5 ml |
| 5 °C ➞ 25 °C, 20 K | 5.0 ml |

   Countermeasure

   While operating at 3000 rpm, cool the rotor to the temperature when the solution is injected to the rotor. Then try to remove the cap again. If it is still difficult, stop running the rotor and push down the push button hard, then remove the cap.

2. When the temperature of Injecting solution is higher than the temperature of the spinning rotor:

   Because of the temperature fall of the solution injected into the rotor the volume decreases and the inside of the rotor becomes a negative pressure status. The cylinder of the cap assembly and the pushbutton are sucked up to the rotor shaft. As the result, the cap cannot be removed. The decreases of volumes are the same values of the increases of volumes shown in the above table.

   Countermeasure

   Remove the cap while operating at 3000 rpm. If still difficult, rise the temperature of the rotor to the temperature when the solution is injected while operating at 3000 rpm and try to remove the cap again.

   Precautions on mounting the seal assembly Wipe the spinning Rulon seal surface with a tissue paper moistened with water lightly just before mounting the seal assembly. This prevents the Rulon seal from wearing abnormally and makes the cap removal easier Keep the seal assembly as horizontal as possible and center it visually. Lower the seal assembly vertically onto the Rulon seal surface and mount it quickly taking care not to hit the adapter on the Rulon seal surface. Never incline the seal assembly when mounting. If the seal assembly is mounted in inclined condition, the tip of the adapter made of Delrin may hit the Rulon surface and the Rulon may be faulty. The flow resistance of extraction pipe must not be large. The diameter of the extraction pipe must not be small and the length must not be long. The flow cell of the UV monitor must not be a micro flow cell .

## 5. 4. 2. Precautions on loading and unloading

1. Do not feed bubbles into the rotor at loading

   » In the pipe connection, provide bubble eliminating equipment to the position just before the seal

   » Be sure to clamp the pipe to keep the no-bubble status up to the exit of the contact body of the seal assembly on all occasions and setting is carried out.

» When the seal assembly is removed due to an injection trouble and the connection is carried out again.

a. When the rotor is full

Inject a heavy solution from “OUTER WALL” side to push the bubbles out of the shaft at ”CENTER” side. Open the end of the pipe for eliminating bubbles and inject a heavy solution (normally 20 to 40 ml) with an injector from “OUTER WALL” side until all the bubbles are eliminated from the solution.

b. When the rotor is not full

Because there is no way to eliminate bubbles from the injections, continue injection. If not, abnormal injection pressure may be applied to the Rulon seal and cause leakage of solution. In addition when containing bubbles not only the density gradients but also the fractionated patterns may be disturbed in the extraction process.

2. Do not feed bubbles into the rotor at unloading

When mounting the seal assembly for unloading after centrifugation, eliminate bubbles at “OUTER WALL” side. Provide the ‘air cushion’, that is an air layer in the pipe for eliminating bubbles to absorb the shock of flow against the seal assembly.

3. Control the pressure for injecting density gradient solution.

» The higher the concentration of solution, the slower the injection speed.

» It is permitted to measure the injection pressure with a pressure gauge to keep the injection pressure at 1 kg/cm² or less.

4. Precautions on mounting the seal assembly

» Wipe the spinning Rulon seal surface with a tissue paper moistened with water lightly just before mounting the seal assembly. This prevents the Rulon seal from wearing abnormally and makes the cap removal easier.

» Keep the seal assembly as horizontal as possible and center it visually. Lower the seal assembly vertically onto the Rulon seal surface and mount it quickly taking care not to hit the adapter on the Rulon seal surface. Never incline the seal assembly when mounting. If the seal assembly is mounted in inclined condition, the tip of the adapter made of Delrin may hit the Rulon surface and the Rulon may be faulty.

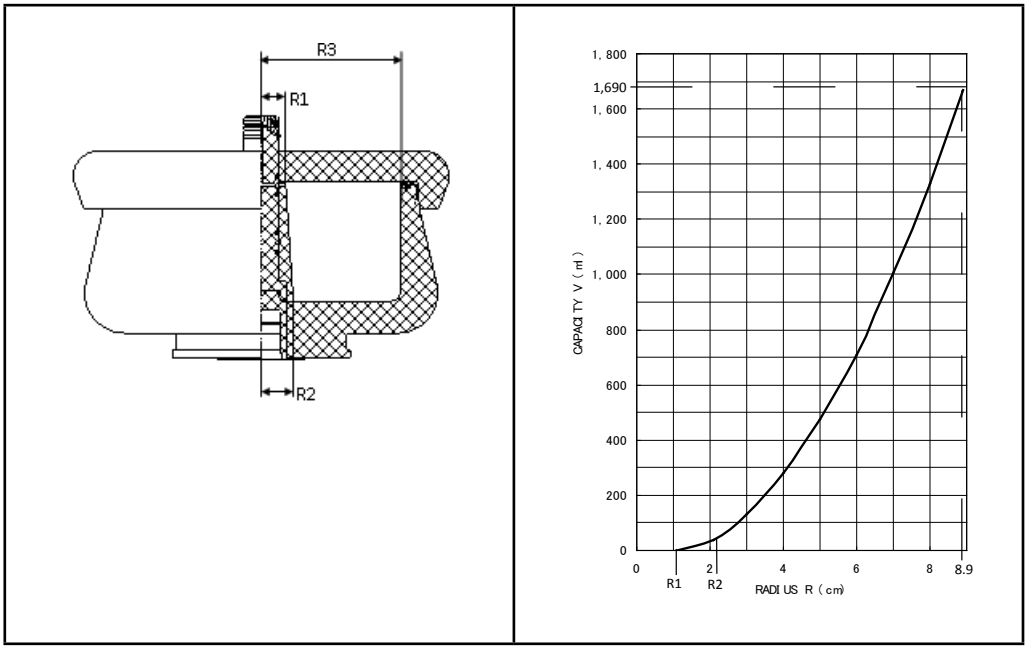
5. The flow resistance of extraction pipe must not be large.

» The diameter of the extraction pipe must not be small and the length must not be long.

» The flow cell of the UV monitor must not be a micro flow cell

## 5. 5. Capacity in Rotor

Figure 38
Relation between volume and radius




## 5. 6. Separation Characteristic

Table 9
Separation characteristic

| Speed (rpm) | (rpm)² | Centrifugal Force (x g) | | | K Factor |
|---|---|---|---|---|---|
| | | $R = R_{min}$ (2.05 cm) | $R = R_{av}$ (5.475 cm) | $R = R_{max}$ (8.89 cm) | |
| 10000 | $1000 \times 10^5$ | 2529 | 6120 | 9940 | 3716 |
| 15000 | $2250 \times 10^5$ | 5160 | 13800 | 22400 | 1652 |
| 20000 | $4000 \times 10^5$ | 9170 | 24500 | 39800 | 929 |
| 22500 | $4840 \times 10^5$ | 11100 | 29600 | 48100 | 768 |
| 25000 | $6250 \times 10^5$ | 14300 | 38200 | 62100 | 595 |
| 27500 | $7563 \times 10^5$ | 17300 | 46200 | 75200 | 491 |
| 30000 | $9000 \times 10^5$ | 20600 | 55000 | 89500 | 413 |
| 32000 | $10240 \times 10^5$ | 23500 | 62700 | 102000 | 363 |

## 5. 7. Rotor Life

**NOTE** Keep the rotor operation log regurlarly. The rotor operation log is necessary for control of the rotor life.

Table 10
Rotor Life

| Operations | Time |
|---|---|
| 1000 | 2500 h |

While using the rotor repeatedly, its strength decreases gradually due to fatigue and creep of material, by which the rotor life is determined. Sum up the number of operations and number of operating hours, and if either value reaches the figures (primary life) shown in Table 7, decrease the maximum speed of the rotor by 10%.

If the operations or operating hours of rotor reach the figures in Table 10 after decreasing the maximum speed by 10% (secondary life), don't use the rotor any more. This specification is applied to the rotor body assembly. The Rulon seal, o-ring, stopper and cap assembly are consumable parts.

# 6. Maintenance

## 6. 1. Cleaning of Rotor and Septa

After use, disassemble and clean the septa, shaft, metal ring, seal packing, and O-ring.

### Cleaning procedure:

Wash it with distilled water at 40 – 50 °C after use, and wipe off water with a soft cloth. After fully drying it, coat it with stopcock grease.

Immediately after a corrosive sample was used, immerse the rotor in neutral detergent (excluding those containing chlorine) diluted with lukewarm water and rinse it sufficiently in distilled water.

### Removal of metal ring

Remove it with the accessory screwdriver or the like which has a thin flat end.

Figure 39
Removal of the metal ring


| | |
|---|---|
| ① | Screwdriver |

## 6. 2. Sterilization of Rotor

Sterilize the rotor appropriately following Table 6-1.

Do not autoclave the rotor or sterilize it in boiling water because its material may deteriorate and its strength may decrease.

Table 11 Sterilization methods

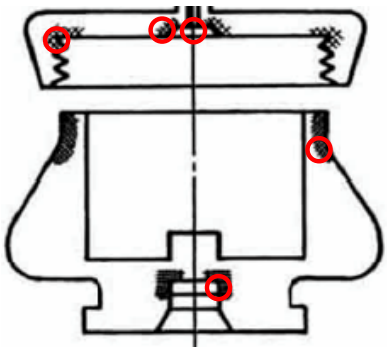
| Sterilize Method | Condition | Rotor |
|---|---|---|
| | | Usable: ✔ Not usable: ✖ |
| Autoclaving | 121 °C (1.0 kg / cm²) for 20 min | ✖ |
| Boiling | 15-30 min | ✖ |
| Ultraviolet rays | 200-300 nm | ✔ |
| Gas | Ethylene oxide | ✔ |
| | Formaldehyde | ✔ |
| Chemical solution | Ethanol (70%) | ✔ |
| | Hydrogen peroxide (3%) | ✔ |
| | Formalin | ✔ |

## 6. 3. Inspection of Rotors

Check the rotor as follows after every use.

- If the optical adapter/disk is unclean, wipe it with a soft cloth but do not damage it.
- Replacements of packings and O-rings are available.
- If the rotor appears to be corroded, deformation, cracks or discoloration is observed, do not use the rotor and call a Thermo Fisher Scientific authorized sales representative. The rotor may be irreparable depending on the degree of corrosion and wear.

Figure 40 Rotor inspection points



# 6. 4. Handling of Rulon Seal and Renewal

Rulon seal is a soft plastic with excellent lubrication developed by mixing tetrafluoroethylene resin with a special filler, and is easily scratched by a fingernail. Do not scratch the upper surface, or solution may leak due to poor sealing.

- Renewal of Rulon seal

  Slightly scratched or worn Rulon seal can be reconditioned by polishing. The flat portion of the zonal rotor cover can used for polishing the Rulon seal. Some technique is required for finishing.

  Put fine sandpaper (No. 800 is recommendable) on a rotor cover, and polish the contact surface until it becomes even.

  Do this carefully while removing polishing agent from the Rulon seal surface and sandpaper. Finish it after making sure no scratch due to the sandpaper remains on the Rulon seal surface.

**Figure 41**
Polishing the Rulon seal

- Removal of Rulon seal

  The Rulon seal can be washed and sterilized together with the shaft. Remove the Rulon seal if the Rulon seal is scratched. Be careful not to scratch the Rulon seal surface.

  Removing procedure

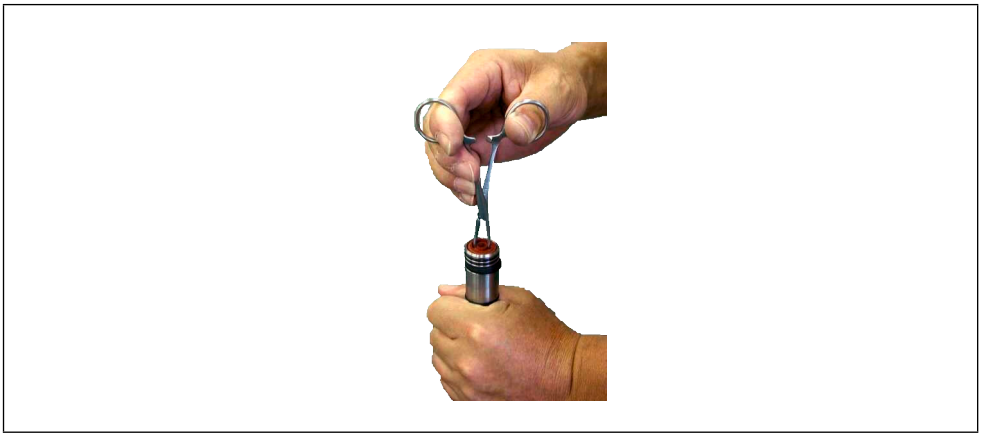
  a. Insert the accessory extractor into two holes.

  b. Turn it clockwise and counterclockwise while lightly depressing it.

  c. Carefully pull it upwards while turning.

     Before resetting it, apply a thin coat of vacuum grease to the O-ring and insert it carefully with soft portions of applied fingers.

**Figure 42**
Removal of the Rulon seal

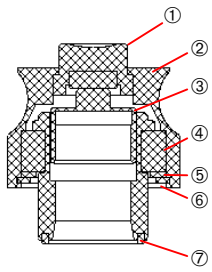


# 6. 5. Disassembling and Cleaning of Cap Assembly

The cap can be disassembled by removing the snap ring using the accessory circlip remover. Be careful not to scratch it since carbon is used as a sliding agent. Wash it in water or clean it by boiling. Figure 43 shows the parts of the cap assembly.

- Check of cap assembly
  - » The cylinder (③) in the cap must move up and down smoothly.
  - » The operation should be almost normal after disassembling and cleaning.
  - » Replace the ring (④) with new one if it has too much play due to wear of ring.
  - » Replace the cap with new one if the corner that catches the stopper is not square.

Figure 43
Parts of the cap assembly




| | |
|---|---|
| ① | Push button |
| ② | Handle body |
| ③ | Cylinder |
| ④ | Ring |
| ⑤ | Washer |
| ⑥ | Snap ring |
| ⑦ | Cylinder case |

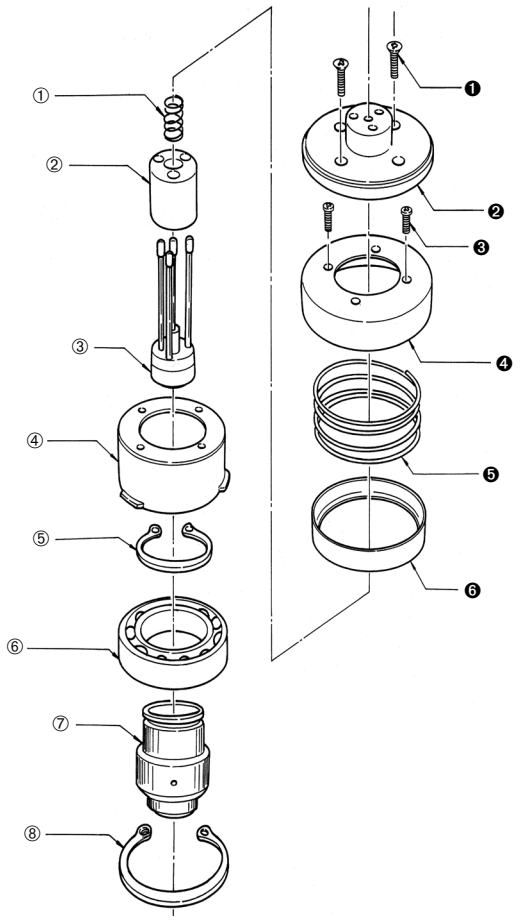
# 6. 6. Disassembling and Cleaning of Seal Assembly

Disassemble the seal assembly by the following procedure. See “Figure 44” on page 46.

1. Loosen screws (❶) and detach the holder cover.
2. Loosen screws (❸) and remove the spring holder, spring (B), and ring.
3. Remove the body, guide body, and coil spring (①).
4. Remove the circlip from the housing, and pull out the adapter and ball bearing.

Fully clean the body, housing and ball bearing. Wash the ball bearing in lukewarm water, dry it, and apply the accessory aluminum lubricant to the balls.

Figure 44
Construction of the seal assembly




| | |
|---|---|
| ① | Coil spring |
| ② | Guide body |
| ③ | Body |
| ④ | Housing |
| ⑤ | Circlip |
| ⑥ | Ball bearing |
| ⑦ | Adapter |
| ⑧ | Circlip |
| ❶ | Screw |
| ❷ | Holder cover |
| ❸ | Screw |
| ❹ | Spring holder |
| ❺ | Spring |
| ❻ | Ring |

## 6. 7. Cleaning of Ball Bearing

Don't leave aqueous sucrose or the other in the ball bearing.

Fully wash it in water or lukewarm water use, and dry it sufficiently with an air blower or dryer.

Slightly apply aluminum screw lubricant. After drying, to the balls to the contacts of the balls with between the inner and outer rings.

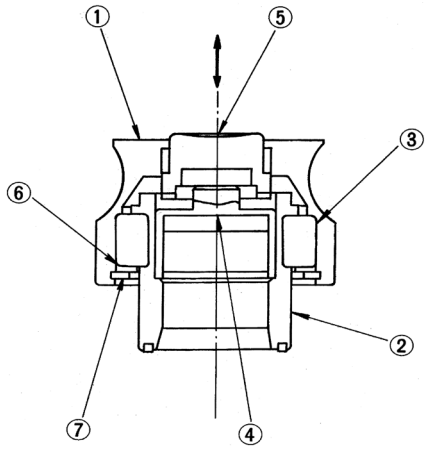
Apply a small amount of lubricant to the inner and outer rings at three positions or so, and turn the bearing so that the lubricant can be applied evenly.

## 6. 8. Consumable Parts of the Rotor

- Rulon Seal
- O-Ring
- Cap assembly
- Seal packing
- Stopper

## 6. 9. Service Parts List for the Cap Assembly

Figure 45
Service parts list for the cap assembly




| No. | Item | Quantity | Article No. |
|---|---|---|---|
| ① | Handle body | 1 | 456310 |
| ② | Cylinder case assembly | 1 | 456132A |
| ③ | Ring | 2 | 458785 |
| ④ | Cylinder | 1 | 456331 |
| ⑤ | Push button assembly | 1 | 456320A |
| ⑥ | Washer | 1 | 450477 |
| ⑦ | Snap ring | 1 | 450483 |

# 6. 10. Service Parts List for the Seal Assembly

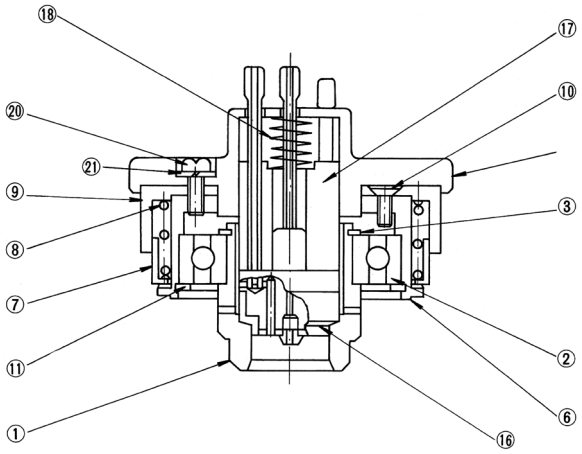



Figure 46
Service parts list for the seal assembly

| No. | Item | Quantity | Article No. |
|---|---|---|---|
| 1 | Adapter (2) | 1 | 481841 |
| 2 | BB | 1 | 60000178 |
| 3 | Circlip | 1 | 481840 |
| | | | |
| 6 | Housing | 1 | 437842 |
| 7 | Ring | 1 | 437844 |
| 8 | Spring | 1 | 437845 |
| 9 | Spring holder | 1 | 437846 |
| 10 | M5 x 10 flat head screw | 2 | 80155598 |
| 11 | Circlip | 1 | 4650213 |
| | | | |
| 16 | Contact body assembly | 1 | 439825A |
| 17 | Guide body | 1 | 437863 |
| 18 | Coil spring | 1 | 452958 |
| | | | |
| 20 | M5 x 12 cross-recessed pan head screw | 2 | 80152408 |
| 21 | M5 spring washer | 2 | 80112058 |

# 7. Troubleshooting

Table 12
Troubleshooting

| Symptom | Possible Cause | Corrective Action |
|---|---|---|
| Pressure is 1.0 kgf/cm² or higher when density gradient solution is injected. | 1. The injection flow rate is too high.<br>2. The injection passage is blocked. | 1. The appropriate flow rate is approx. 50 ml/min.<br>2. Disassemble and check. Blocking is likely to occur in the seal assembly, shaft and septa. |
| Bubbles enter the sample line. | 1. The pressure rises.<br>2. The solution stops flowing. | 1. If the pressure does not rise much, it is possible to continue operation.<br>2. If the pressure is too high or the sample stops flowing, remove bubbles. See “5. 4. 2. Precautions on loading and unloading” on page 39. |
| Pressure drops abruptly during loading and unloading. | The sample injection line is bypassed. | See “Table 6” on page 39. |
| Sample leakage and vibration of seal assembly. | 1. The installation condition of the guard plate.<br>2. The condition of the seal assembly. | 1. Level the guard plate.<br>Rattle the three supports.<br>Center the rotor and guard plate. See “5. 2. Preparation” on page 23.<br>Adjust the height of rotor and case.<br>2. Check the surface of the contact body for the Rulon seal for scratches and wear.<br>Check the bonded portion of the rear of the contact piece for peeling.<br>Check the adapter inside for scratches and wear.<br>Spins the bail bearing smooth? Clean and grease the bail bearing. |
| Temperature of the rotor rises. | Poor degree of vacuum. | Precool the rotor body and sample to separate at low temperatures. See “5. 4. 1. Setting of sample temperature and rotor temperature” on page 39. |

Table 13
Defective phenomena and corrective action sealed sections

| Symptom | Location | | Probable Cause | Corrective Action |
|---|---|---|---|---|
| | Part Name | Portion | | |
| Bypassing and leak of sample and solution. | Seal assembly (Contact body) | WC sealing face (in contact with seal) | 1. Poor sealing due to surface roughness or injury. | Replace the contact body assembly. |
| | | | 2. Poor follow-up in vertical direction of contact body assembly with seal. | Check the spring which depresses the guide body. |
| Bypassing and leak of sample and solution. | Rulon seal | Contact face | Poor sealing due to surface roughness, injury or step between inner and outer rings. | Replace the Rulon seal. |
| Bypassing | | Insertion recess for O-ring | Poor sealing due to injury in recess portion in contact with O-ring. | Replace the Rulon seal. |
| | | O-ring (1AP3) | Poor sealing due to degradation or injury. | Replace the O-ring. |
| Outer wall line solution line leaks | | O-ring (1AP14) | 1. Poor sealing due to degradation or injury. | |
| | | | 2. Poor sealing due to insufficient grease. | Apply grease. |

# 7. 1. Check the Rulon Seal

There are most problem occurrences of Rulon seal in the defect in the operation of zonal rotor.

Explain the point of the check of Rulon seal.

- Check whether the Rulon seal is faulty or not
  - » Scratches or dents on surface
  - » Abnormal wear on surface due to previous sample leakage

  Is the height of the surface of the outside circle and the surface of the inside circle the same?

**CAUTION** Be extremely careful when removing the Rulon seal because the removal of the Rulon seal from the rotor may influence the damage (deformation) to the Rulon seal greatly.

When mounting a new Rulon seal, check it according to the above procedure.

- Check of Rulon seal for stable placement

  The O-rings must be mounted to the perimeter and the bottom of the Rulon seal correctly.

  When mounting the Rulon seal to the rotor shaft, press the Rulon seal comparatively hard so that it is mounted deeply without inclination.

  Apply a thin coat of vacuum grease to both the big and small O-rings.

# 7. 2. Other Check Points

- Check of O-rings of rotor shaft for wear

  Remove the O-rings and check them for wear or crack by stretching a little. If worn or cracked, replace the O-rings with new ones.

- Wear or elongation of stopper

  Check the lower corners of the stopper for wear to prevent the cap from falling off.

  Replace the stopper with new one every six to twelve months.

- Check of other O-rings mounted to the shaft for scratch

  Be careful when inserting the shaft into the septa because the O-rings are susceptible to scratches and cuts. Before inserting. apply a thin coat of vacuum grease to the shaft and O-ring for smooth insertion.

- Check of sample passage of septa for plugging

  Insufficient maintenance after use may result in a partial plugging.

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