TCF-20 Rotor

Specifications

Description	Specification in the Thermo Scientific™ Sorvall™ Lynx 6000 Superspeed Centrifuge
Weight of empty rotor	7.80 kg (17.2 lbs)
Total capacity (nominal)	1,350 mL
Maximum speed	20,000 rpm
Critical speed	4,200 rpm
Max. / Min. / Avg. radius	96.0 mm / 36.0 mm / 66.0 mm
Maximum RCF value at r_{max} / r_{min} / r_{avg}	42,931 x g / 16,099 x g / 29,400 xg
Diameter	213 mm (8.40 in)
Design load	1350 mL at 1.2 g/mL
K-factor at maximum speed	620

Installing tubing

- Open Inlet ports – push open rubber plugs



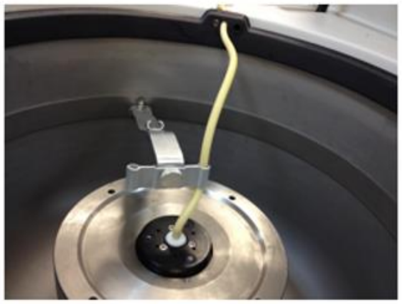
- Place Tube Holder Assembly




Tube Installation by Mode

- Zonal Sealed:

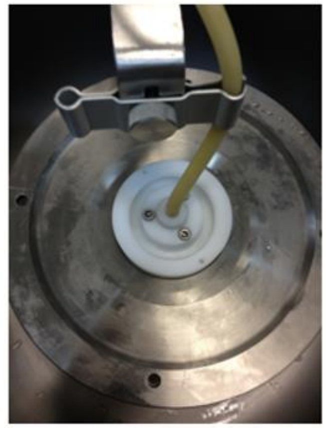
During loading and unloading of the rotor



- Continuous Flow

- Zonal Un-Sealed

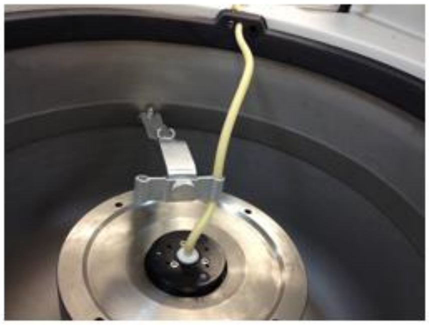
During loading and unloading of the rotor



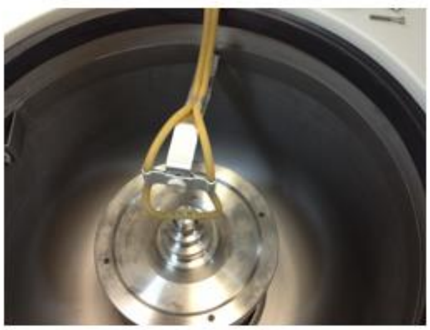
Tube Installation by Mode

- Zonal Sealed:

During loading and unloading of the rotor

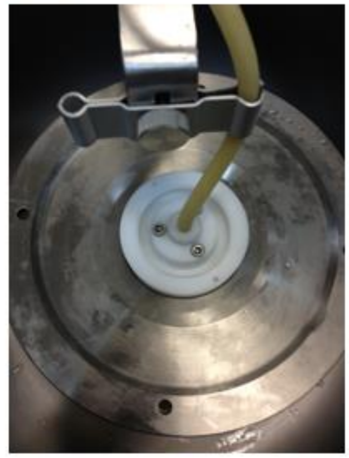


- Continuous Flow



- Zonal Un-Sealed

During loading and unloading of the rotor

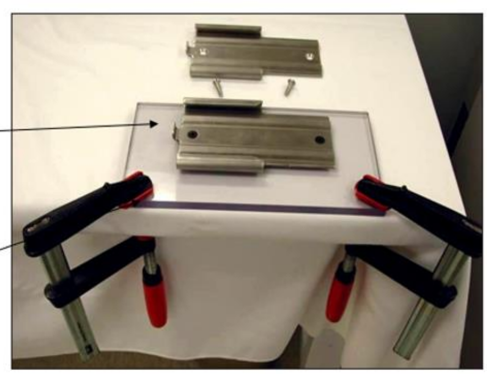


Install rotor holding fixture

- Secure rotor holding fixture to bench
  - Secure directly to bench top with screws

OR

  - Screw onto a block of plastic, wood or metal
    - Secure with clamps
    - Move easily



Install drive adapter

- Place Superspeed Drive Adapter onto center of rotor

- Secure with three cap screws using hex wrench

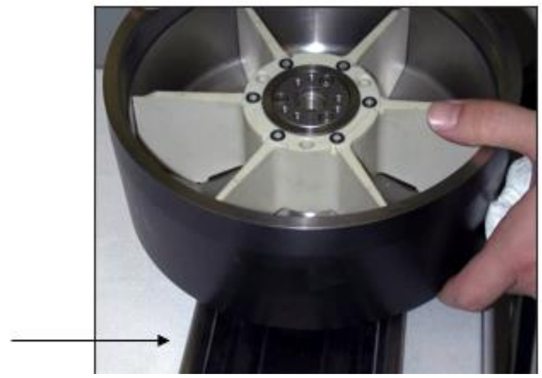
Insert Septa

- Ensure O-rings are lubricated and in place

Secure rotor onto holding fixture

Slot on rotor base

- Secure rotor base onto rotor holding fixture

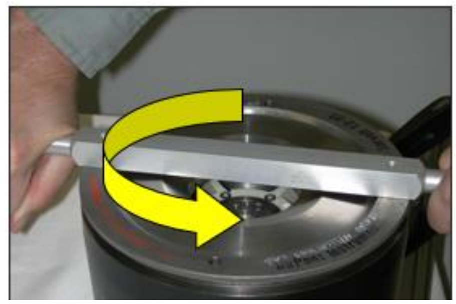


Fill rotor

- Fill the rotor with sample, buffer or water
  - To avoid damage to the septa, the rotor must never be run empty

Tighten rotor cover

- Be sure rotor cover o-ring is in place & lubricated with vacuum grease
- Lubricate threads with tan grease and place on rotor body
- Tighten cover
  - Use rotor wrench
  - Turn counter clockwise
  - Using effort, tighten cover completely until rotor tightens to stop point. Tighter is better!
    - If cover is too loose, o-ring may reposition during run and “freeze” cover to body



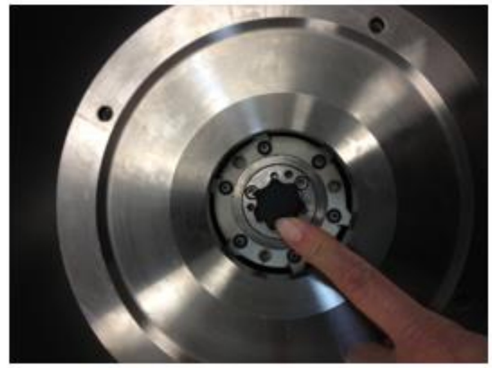
Install rotor on spindle

- Place rotor onto drive spindle
- Rotor automatically secured – AutoLock

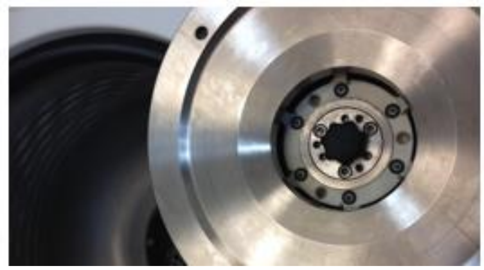
- Rotor automatically identified- AutoID

Removing the rotor

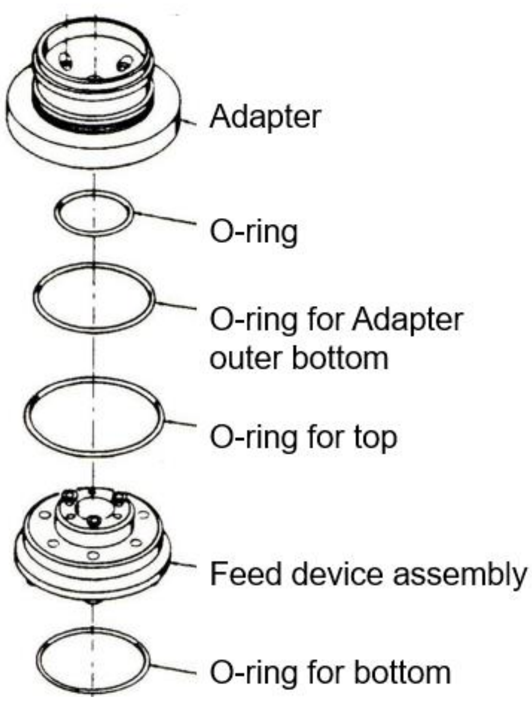
- Place the unlock tool into the center and push down to release AutoLock



- Lift and remove the rotor



Install Continuous Flow Adapter Assembly



Continuous flow
Adapter Assembly

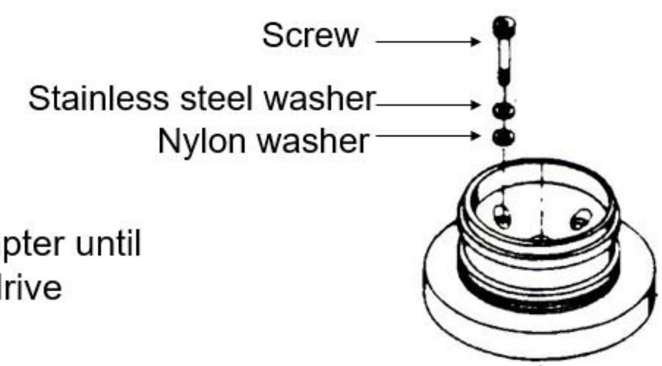
- Slip the adapter over feed device
- Compress to mate

Install continuous flow adapter

- Place assembled continuous flow adapter through center opening of rotor cover

- Rotate to engage guide pins into holes of septa

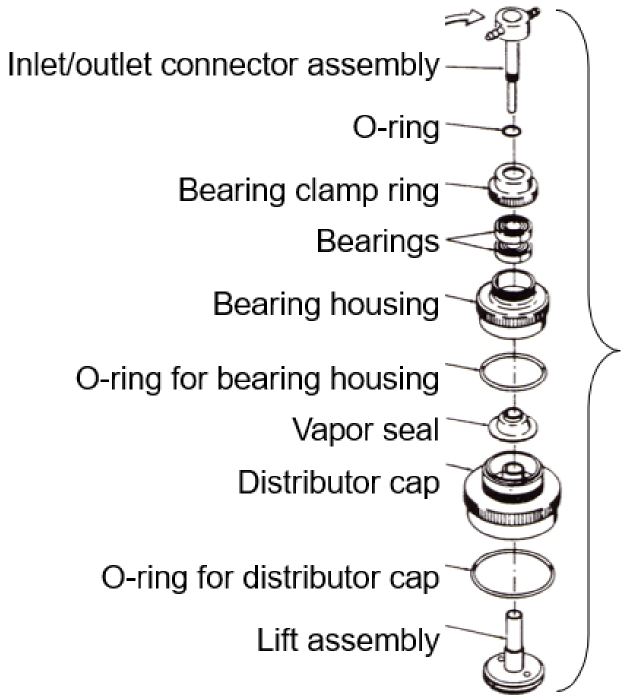
Install continuous flow adapter



- Rotate continuous flow adapter until screws align with holes in drive adapter

- Tighten screws evenly using hex wrench

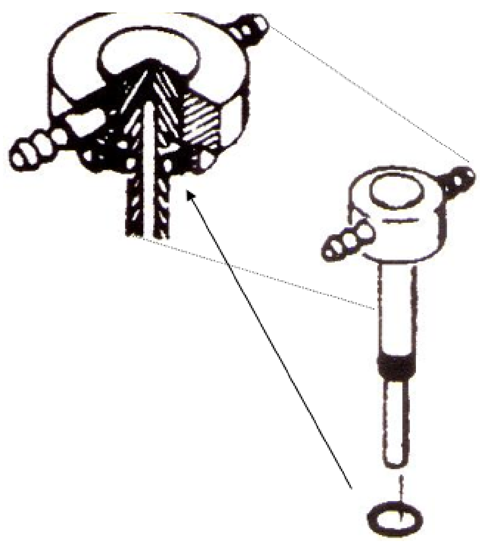
Install inlet/outlet assembly



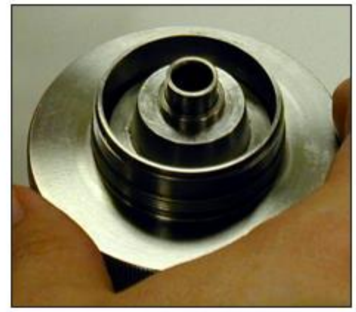
Inlet/outlet assembly

Install inlet/outlet assembly

- O-ring
  - Located in inlet/outlet connector
  - Undergoes relatively little stress
  - Removal can be difficult & cause damage to the o-ring
  - Replace only if damage or wear is suspected



Install inlet/outlet assembly



Lift assembly
Distributor cap

Vapor seal

Install inlet/outlet assembly

Bearing grease
Bearings

Small "dots of grease between bearings

Bearing housing

Ball Bearings face each other

Bearing clamp ring

Turn *counter*-clockwise by hand until tight.

Install inlet/outlet assembly

Install assembled bearing housing on distributor cap. Turn *counter*-clockwise by hand until tight.

Inlet/outlet connector assembly

Tighten lift assembly with lift plate wrench Turn *counter*-clockwise

Inlet/outlet assembly

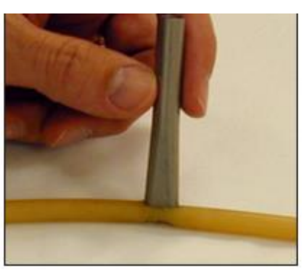
Turn *counter*-clockwise by hand until tight.

Inlet/outlet connector assembly should spin smoothly

Inject sealing fluid

Use 3-5ml syringe & 20 G needle. Inject ~3 ml of 85% glycerin into small hole in bearing housing

Install tubing



Punch hole in tubing

Install tubing over inlet/outlet connector

Push excess tubing to sides

^ ^ Use hammer and tube slotter to punch hole in center of latex tubing

Set up peristaltic pump

- Power source at back of instrument
- Install tubing through peristaltic pump
- Place outflow latex tubing in a collection vessel located below rotor level
- Pump can only be operated at 8,000 rpm
  - – No power at rpm < 8,000 rpm

Start the run

- Proper start-up sequence
- Proper start-up sequence
  - Install complete system and tubing
  - Close centrifuge door
  - Set desired centrifuge speed
  - When set speed is reached ($\geq$ 8000 rpm), turn on peristaltic pump to introduce sample.
    - Sample may leak from rotor assembly into chamber if peristaltic pump is turned on before rotor speed reaches 8,000 rpm.
  - Check for cleared supernatant from the outflow tubing (may take a few minutes before all liquid takes up final “dead-volume” in rotor).
- **NEVER RUN WITH ROTOR EMPTY**

Evaluate clearance & operation during run

- To increase pelleting efficiency and increase supernatant clarity:
  - Decrease speed (flow rate) for peristaltic pump
  - Increase rotor speed up to 20,000 rpm
- Stop the run
  - In event of abnormal sounds or vibrations
  - If the supernatant suddenly becomes cloudy (maximum pellet achieved)
  - If outflow ceases or slows (line blockage, rotor leakage, all sample processed)

Stop the run

- Proper shut-down sequence
  - Turn off peristaltic pump and clamp tubing
  - Then stop the centrifuge run.
    - To prevent leakage, never operate peristaltic pump when rotor is spinning below 8,000 rpm.
  - At 0 rpm, open centrifuge door
  - Remove tubing holder assembly
  - Remove inlet/outlet assembly
  - Unscrew rotor from drive spindle
  - Remove rotor from instrument

Disassemble rotor & remove product

- Gently slide rotor onto rotor holding fixture
- Use wrench to remove rotor cover by turning *clockwise*
- Pour off excess liquid -or- draw it out with large syringe or peristaltic pump
- Remove septa
- Use spatula or cell scraper to remove packed pellet

Rotor cleaning & care

- After operation, disassemble rotor and each assembly
- Remove any heavy spills or encrusted material
- Use polyethylene squeeze bottle to flush clean any lines or ports
- Wipe away excess grease from metal threads
- Soak and wash parts in mild laboratory detergent (Avoid harsh or abrasive cleaning methods)
- Check chemical compatibility charts (in rotor manual) when using disinfectants & cleaners
- Rinse parts in water, then dry
- Do not immerse bearings in water
- Clean bearings in toluene, then dry ball-side down
- The rotor, lid and metal parts can be autoclaved. (Avoid autoclaving the bearings, o-rings, septa and GK feed device assembly)
- After parts are completely dry & inspected, re-lubricate using appropriate greases, then re-assemble and store

Disassemble inlet/outlet assembly

- Insert pins of lift plate wrench into corresponding holes of lift plate assembly

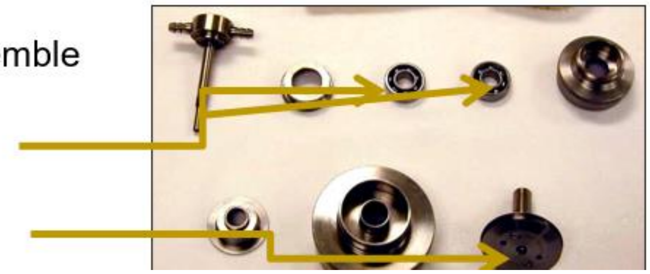
- Grasp arms of inlet/outlet connector
- Turn *clockwise* to unscrew

- Turning *clockwise*, remove bearing housing from distributor cap

- Then, remove bearing clamp ring from bearing housing
- If necessary, use strap wrench

- Remove bearings from bearing housing.
  - The strap wrench handle makes an excellent tool to push out bearings

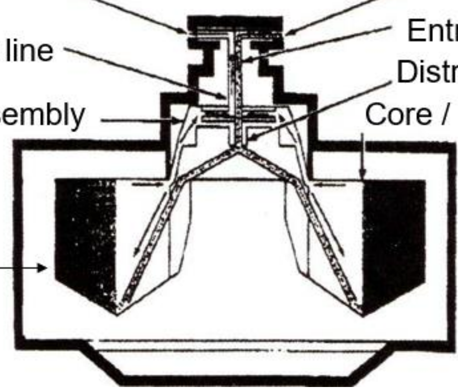
- Clean, inspect & reassemble
- Keep pieces together



Inspect inlet/outlet assembly

- O-rings
  - Ensure all o-rings are in place & in good condition
  - Replace if necessary
  - Lubricate with vacuum grease
- Metal threads
  - Wipe away excess, old grease from threads
  - Lubricate with tan grease
- Bearings
  - If necessary, clean in toluene & dry ballside-down
  - Lubricate with a small amount of red grease between each ball (see rotor manual for details)
  - Discard & replace worn, rusted or heavily encrusted bearings

Theory of operation

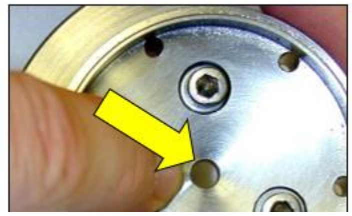


Liquid flow path

- The inlet/outlet and continuous flow adapter assemblies sit atop the septa
- Fluid enters the “In” arm of inlet/outlet connector assembly

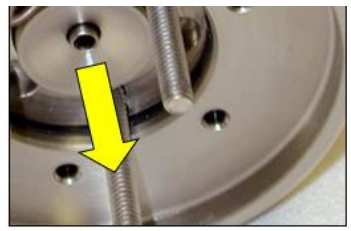
- Flow proceeds down through “stalk” of inlet/outlet connector assembly

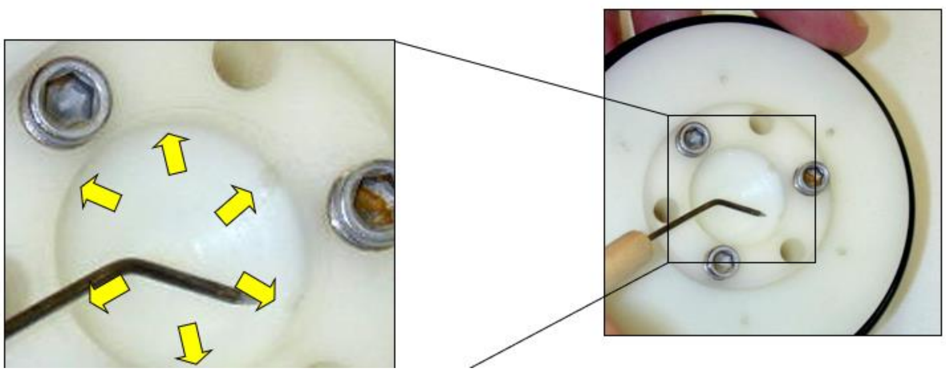
- and the “stalk” extends down through center hole of the adapter



- Flow continues down through the six small holes in center portion of feed device assembly

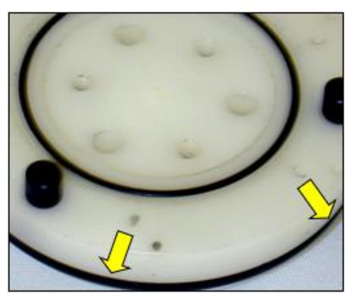
Bottom view



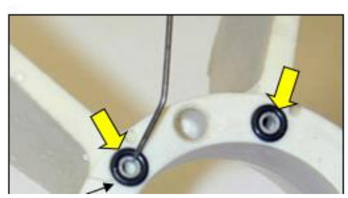


- …and out through innermost holes on outer periphery of GK feed device assembly

Bottom view

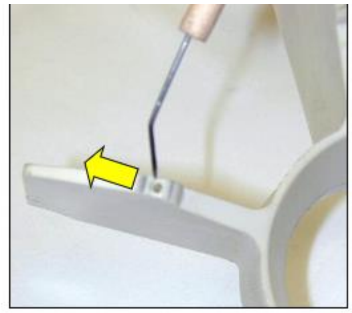


- The pegs on bottom of GK feed device assembly line up with holes in septa
- When connected, fluid flows into holes of septa lined with o-rings.



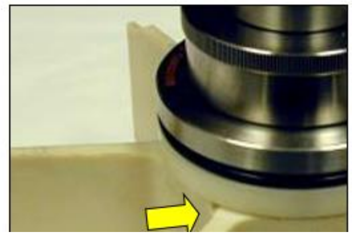
- Fluid flows through channels in septa arms and out through hole in bottom middle portion of each arm

Bottom view



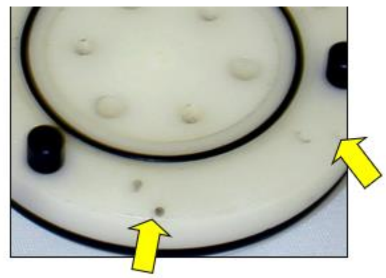
- Particles pellet on wall of rotor body

- Cleared liquid supernatant is channeled up through small holes located just left of each septa fin

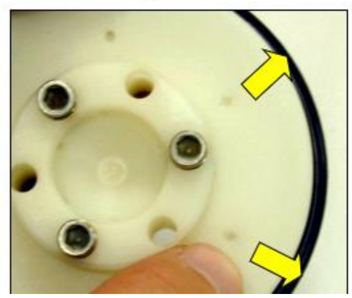


- Supernatant then travels up through outer holes in bottom of GK feed device assembly

Bottom view

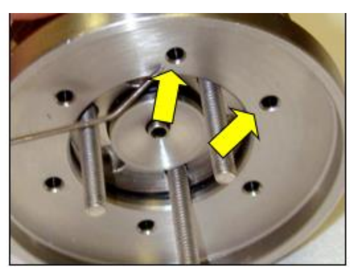


- …and exits through the holes on top

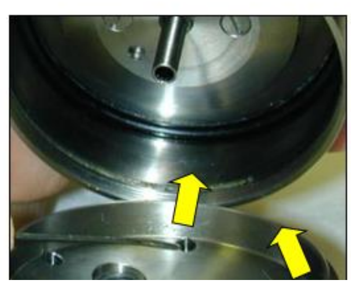


- The liquid is further channeled into the six outer holes in bottom of the adapter

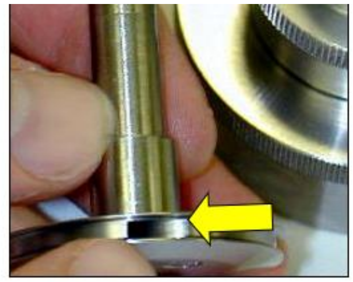
Bottom view

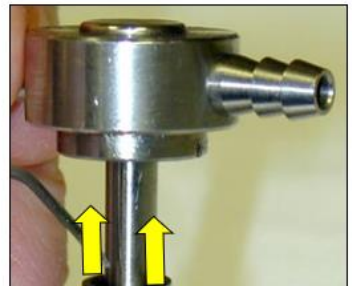


- … and up into the cavity between the adapter and inlet/outlet assembly



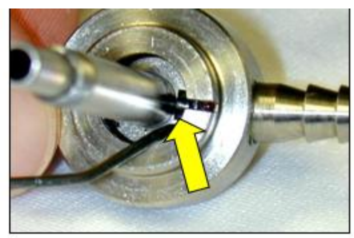
- The lift assembly draws liquid…
- ... which travels up through space between lift assembly & inlet/outlet connector “stalk”

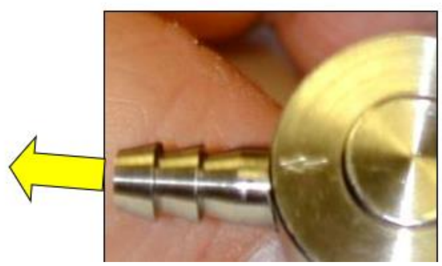




- Lift assembly channels liquid to outflow channel seen underneath inlet/outlet connector assembly
- Finally, cleared supernatant exits the outlet

Bottom view





Common Questions & Answers

- How do I know if the bearing have been flooded? Flooded bearings will contain liquid, and the bearing grease may turn pinkish-white, rather than the normal red color.
- How should I treat flooded bearings? Clean the bearings in toluene, then invert to dry. Blast out any residual liquid with an air spray can. Re-lubricate the bearings with red bearing grease as per instructions in rotor manual.
- What is the danger of flooded bearings and how can I prevent this? Flooded bearings are more likely to cause lock-up of, or leakage from, inlet/outlet assembly. Ensure the bearings are in good shape, not flooded and properly lubricated. Reduce back-pressure by turning down peristaltic pump speed and keeping outflow line below rotor level.
- How often should I replace the bearings (General guidelines)? Replace bearings after heavy use at high rotor speeds. For example, if processing 20-30 L at max speed, replace bearings every 1-2 runs. If spinning 6-10 L at only 12,000 rpm, bearing life is prolonged to 5-10 runs.

- Can I autoclave the rotor or steam-in-place (SIP)? The rotor, lid and metal parts can be autoclaved. Avoid autoclaving the o-rings, bearings, septa and GK feed device assembly. The system is not designed for SIP
- How can I calculate time for separation? Time=sample volume÷flow rate (e.g. 10,000mL÷200mL/min = 50min). The run will take longer If the sample volume exceeds maximum rotor capacity (see below).
- How do I estimate volume separated before reaching maximum pellet? Centrifuge a small volume of sample via standard rotor to determine grams of pellet per sample volume. Then, multiply by total volume of sample. For example: Customer has 8,000mL of sample. Spinning 50mL of sample in standard centrifuge tube results in a 5 mL pellet at bottom (0.1mL pellet/mL sample). Then calculate 8,000 mL x 0.1 = 800 mL. Thus, this 8L sample will match maximum rotor capacity, which is only 800 mL of pellet. -OR- take an O.D. spectrophotometer reading of sample. Convert O.D. to concentration (specific for particle), then multiply concentration x volume of sample.

- What is the appropriate rotor speed and pump rate to separate a certain particle?

  The pump rate and rotor speed depend greatly upon particle sedimentation coefficient, density & size. If supernatant from outflow is cloudy, reduce pump speed &/or increase rotor speed until sample is cleared In practice the following guideline may help: Start with a flow rate of 200mL/min and a rotor speed of 15,000 rpm. Collect supernatant aliquots and assess for clarity (visual, spectrophotometry, etc). If clear, increase pump rate until particles start to come out supernatant. Then, back down pump speed slightly. It may not be necessary to run rotor and pump at top speeds. (lower rotor & pump speeds prolong bearing life and reduce chance for leakage)

- The supernatant looks slightly cloudy, but clears up within 20-30 seconds. What's going on? The supernatant may contain tiny bubbles that rise to the surface within a minute after collection, clearing the sample.
- Can anything be done to prevent excessively foamy supernatant? Reduce or eliminate detergents from the sample. Some biological fluids naturally foam during continuous flow separation. In such cases, the optional high speed lift assembly may help reduce foaming.
- The density of the solution I wish to separate exceeds 1.2g/ml. Are there any specific precautions I should take? Yes. If suspension has specific gravity > 1.2 g/cm^3, the depth of pelleted sediment must be limited for safe operation. See Rotor Manual for details.
- What is the outer and inner diameter of the tubing? O.D. 5/16", I.D. 3/16"
- Instead of slotting the tubing and stretching it over the inlet/outlet connector assembly, can I cut the tubing and attach it with ties? We do not recommend this process, because tied-on, cut tubing is more likely to pop-off and flood the centrifuge chamber.

- Can I use one type of tubing rather than splicing latex (inlet/outlet) with silicon (peristaltic pump)? We have successfully tested tubing (such as PharMed) that is elastic enough to stretch over inlet/outlet, yet durable enough for the peristaltic pump. This allows one continuous line of tubing, rather than splicing. However, latex tubing is not compatible with peristaltic pumps, and silicon is not sufficiently stretchy for the inlet/outlet.
- How can I pellet the material left in tubing at end of run? Pump water, buffer or medium until all sample exits the tubing and enters the spinning rotor.
- How do I calibrate my peristaltic pump? Set pump to a particular numerical setting. Place the inlet tubing in sample (or buffer, water, etc) and the outlet tubing in an empty graduated cylinder. Turn pump on and use a stopwatch to determine the volume of liquid collected per unit of time. Repeat this for several pump settings. Post the calibration near the pump for easy reference

Troubleshooting

- Rotor cover difficult to remove after run: Be sure cover is *completely* tightened onto body. Tighter is better (prevents o-ring from re-positioning during run). Carefully wipe-away all old grease from threads of cover and body. Re-lubricate with tan grease. Lubricate cover o-ring with vacuum grease.
- Inlet/outlet assembly pieces difficult to dissasemble: Use strap wrench to disassemble, if necessary. Clean old grease from metal threads and re-lubricate with tan grease. Lubricate all o-rings with vacuum grease. Do not over-tighten assembly (hand-tight is sufficient)
- Bearings flooded: Eliminate any occlusions in outflow line that create back-pressure. Keep outflow tubing below the level of the rotor. Reduce peristaltic pump speed. Do not operate peristaltic pump when rotor is spinning below 8,000 rpm. Clean, dry & re-lubricate with bearing grease.
- Inlt/outlet assembly locks-up during run: Ensure bearings are in good shape, lubricated & not flooded. Ball bearings must face each other. Check for proper assembly, and spin the top connector “T” by hand before running to make sure it spins freely and smoothly. Check parts for damage & re-order if necessary.

- After turning off peristaltic pump, fluid still flows from outlet tubing: Clamp off tubing to prevent any siphon effect. Adjust pump rollers so they press sufficiently against tubing.
- Liquid in chamber after run: Probable leakage from inlet/outlet assembly. To prevent: Reduce peristaltic pump rate. Do not operate peristaltic pump when rotor spinning below 8,000 rpm. Check for holes in tubing and for proper tubing installation. Use 85% glycerin as final sealing fluid. Eliminate any occlusions in outflow line that create back-pressure. Keep outflow tubing and supernatant carboy below the level of the rotor.
- Outflow (supernatant) is cloudy, contains particles: To increase pelleting efficiency, decrease pump rate and/or increase rotor speed. Also, maximum pellet capacity may be exceeded, causing uncleared sample to exit the rotor.