

# Corning® Glass Spinner Flasks

## Operating Instructions

4500, 4502, 4510, and 4512 Series

CORNING



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# Scientific Support and Safety Information

## Scientific Support

For technical information, visit [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

## Safety Instructions

The following precautions should be followed any time you work with glass vessels. Safety glasses should be worn at all times. Take extreme caution whenever you handle glassware, especially when washing and assembling/disassembling glass spinner flasks. We strongly recommend that the flask is replaced if any cracks or chips appear anywhere on the vessel, center neck cap or sidearms. Under no circumstances should the vessel be pressurized or evacuated without precautions.

## Safety Information for Glass Reservoirs

Since glass reservoirs cannot be guaranteed for any positive or negative pressure, evacuated, or pressurized glass reservoirs must be shielded.

The Corning® Glass Spinner Flasks described in this instruction manual all include at least one glass reservoir. Glass has traditionally been the material of choice for spinner flasks, because it is relatively inexpensive, easy to clean, provides high visibility of reservoir contents, and is autoclavable. Many researchers sparge their vessels, or provide gas overlays to improve oxygenation of the flask medium. Even if there is no intent to pressurize a reservoir, there is the chance of accidentally pressurizing a glass reservoir during gassing if the sidearm caps are tightened or a vent filter becomes blocked. Special precautions should therefore be taken. It is not possible for a manufacturer of glass reservoirs to guarantee that its reservoirs will hold any specific positive or negative pressure without breaking.

For this reason, Glass Spinner Flasks should always be shielded if gassing of the vessel is attempted. In addition, good laboratory procedures should always be followed in handling and cleaning glassware. The use of personal eye protection should be routine. See below for a full statement of limitation of liability regarding Corning glass reservoirs.

## CAUTION



Corning laboratory glass is intended for laboratory use only. The well-being of individuals using these products is dependent on their proper operation, maintenance and safe laboratory practices. Refer to the owners guide, product package inserts, and labels concerning warnings, safe use, and operation of any Corning laboratory product.

# Introduction

For optimum performance and safety, please read these instructions carefully.

## Glass Spinner Flask Models

This manual provides instructions for assembly and operation of the Corning Glass Spinner Flasks: 4500, 4502, 4510, 4512 Series

There are 2 basic styles of Corning Glass Spinner Flasks: angled sidearms and vertical sidearms.

### Angled Sidearms, 4500 Series

Provided with a center neck cap assembly and an impeller assembly for magnetically driven stirring

Working Capacity	125 mL	250 mL	500 mL	1L	3L	6L	8L	15L	36L
Cat. No.	4500-125	4500-250	4500-500	4500-1L	4500-3L	4500-6L	4500-8L	4500-15L	4500-36L
Center Neck (mm)	70	70	100	100	100	100	100	100	100
Sidearm Caps (GL)	32	32	45	45	45	45	45	45	45

### Angled Sidearms, 4502 Series

- Two sidearms angled at 45°
- 120 mm center neck cap and GL45 sidearm caps
- Provided with a center neck cap assembly and an impeller assembly for magnetically driven stirring

Working Capacity	125 mL	250 mL	500 mL	1L	3L	6L	8L	15L	36L
Cat. No.	--	--	--	--	4502-3L	4502-6L	4502-8L	4502-15L	4502-36L

### Vertical Sidearms, 4510 Series

- Vertical sidearms (4 in 8L and 15L capacities, 6 in 36L capacity)
- 100 mm center neck cap and GL45 sidearm caps
- Not provided with an impeller assembly
- Center cap assembly provided does not include details for using an impeller assembly
- Center cap assemblies and impeller assemblies for magnetically stirring or for direct drive motor stirring are available separately

Working Capacity	125 mL	250 mL	500 mL	1L	3L	6L	8L	15L	36L
Cat. No.	--	--	--	--	--	--	4510-8L	4510-15L	4510-36L

### Vertical Sidearms, 4512 Series

- Vertical sidearms (4 in 8L and 15L capacities, 6 in 36L capacity)
- 120 mm center neck cap and GL45 sidearm caps
- Not provided with an impeller assembly
- Center cap assembly provided does not include details for using an impeller assembly
- Center cap assemblies and impeller assemblies for magnetically stirring or for direct drive motor stirring are available separately

Working Capacity	125 mL	250 mL	500 mL	1L	3L	6L	8L	15L	36L
Cat. No.	--	--	--	--	--	--	4512-8L	4512-15L	4512-36L

# Getting Started

## Cleaning and Siliconizing Your Vessel

### Standard Glassware Cleaning Protocol

Although you should find your glass vessel and components to be clean in appearance as received, Corning does not perform any cleaning processes on the components prior to shipment. Corning strongly recommends that you wash the vessel and components prior to use.

All components should be washed using a laboratory detergent followed by thorough rinsing with distilled or deionized water.

**CAUTION:** Use only cleaning solutions that do not leave residue that could hinder the cell culturing objective.

Any brushes used during washing should be made of plastic rather than metal.

### Siliconizing Glassware

The basement membrane produced by many attachment-dependent cell lines grown on microcarrier beads may adhere to the glassware where it can complicate subsequent cell culture. In addition, proteinaceous material from cells or media may accumulate at the gas-liquid interface, creating a “crust” that can encourage further deposits.

It is best to minimize its buildup on the wall of the vessel by placing a layer of silicone on the glassware. A layer of silicone can be created on the glass vessel, using siliconizing products marketed for that purpose and following the instructions provided by the selected product's manufacturer.

Be sure to thoroughly wash the vessel after treatment prior to its use in cell culture. One treatment is usually sufficient for many cell culture cycles. A follow-up treatment is necessary when a buildup of cells or microcarriers on the surface of the glassware is noted.

The protocols described above are general procedures which work well with glassware that has had little use. In the event that the vessel has been used extensively and is markedly soiled, please refer to the section “Disassembly, Care, and Maintenance” prior to siliconizing the glassware.

## Assembly of Vessels and Components

### Impeller Assemblies for Magnetically Driven Glass Spinner Flasks

Impeller assemblies for magnetically driven glass spinner flasks are provided with the following components:

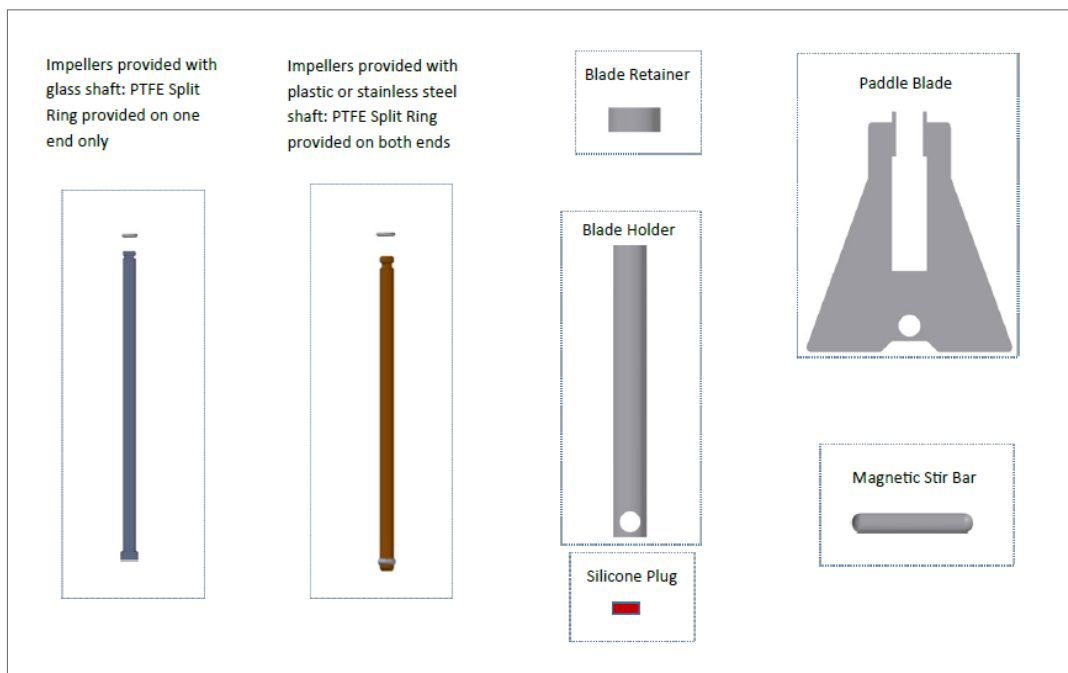


Figure 1. Corning Spinner flask impeller components.

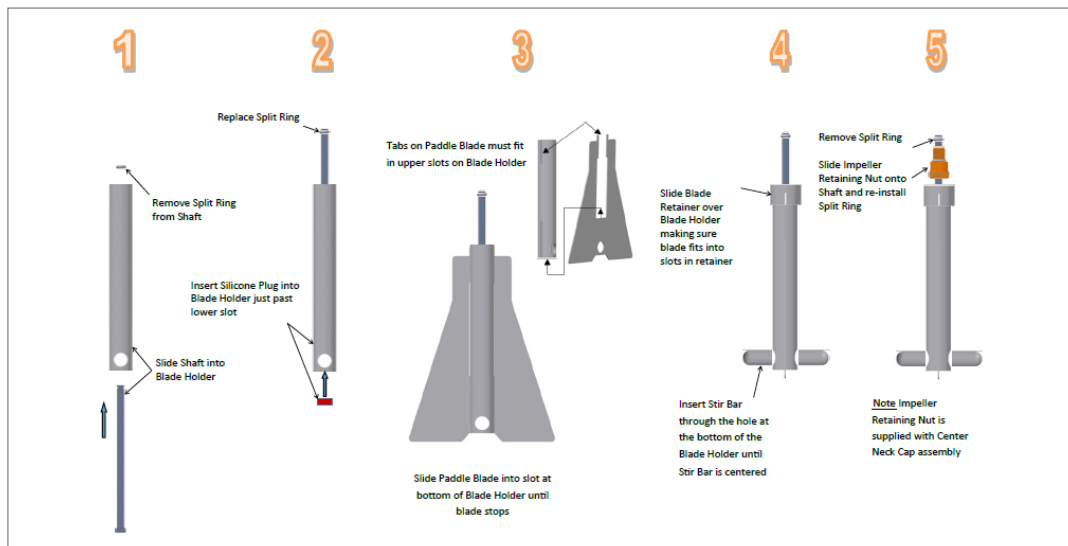


Figure 2. Corning Spinner Flask Impeller assembly steps.



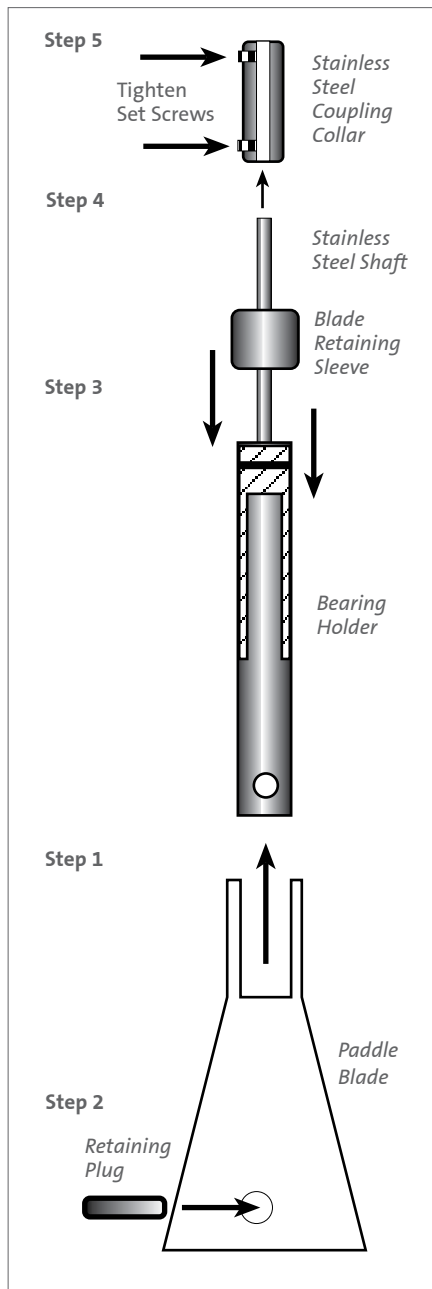


Figure 3. Direct drive paddle assembly

### Impeller Assemblies for Glass Spinner Flasks Driven by Direct Drive Motors

Direct Drive Impeller Assemblies are assembled as shown in the figure to the left of this page and per the following instructions.

1. Slide the Paddle Blade into the notch in the Bearing Holder (narrow end of the Paddle Blade first) until the hole on the blade lines up with the hole in the bearing holder.
2. Insert the short, rounded Retaining Plug into the aligned holes until it is centered in the combined assembly. The Paddle Blade should not be able to slide on the Bearing Holder.
3. Take the Blade Retaining Sleeve with the notched end facing down and slide it down on top of the newly assembled Bearing Holder and Paddle Blade. Position the narrow ends of the Paddle Blade into the notches of the Blade Retaining Sleeve and push down until the top portions of the blade extend completely into the notch in the retaining sleeve.
4. Slide down one end of the Stainless Steel Coupling Collar onto the Stainless Steel Shaft. The other part of the coupling collar will slide up onto the flat portion of the drive unit shaft in a later assembly. For more detail, see Figures 4 through 7.
5. Tighten the set screws on both sides of the collar so that the collar does not turn on the shaft.



Figure 4. Coupling collar – larger opening for paddle shaft



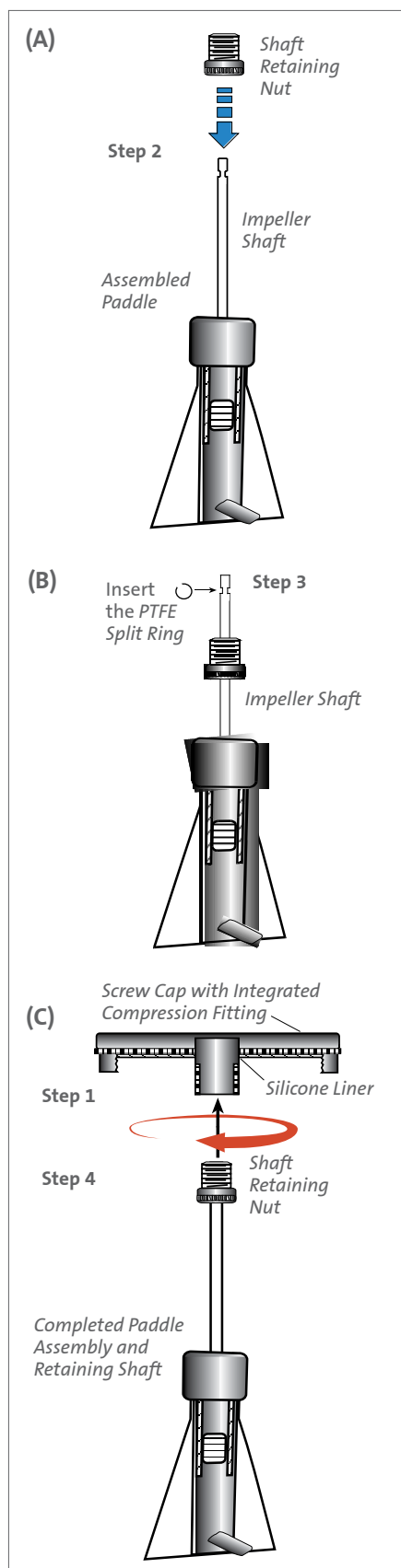
Figure 5. Coupling collar – smaller opening for drive shaft



Figure 6. Slide larger opening on coupling collar over paddle shaft (Step 4).



Figure 7. Tighten lower set screw (Step 5).



**Figure 8.** Magnetically driven screw cap assembly

## Center Cap Assemblies for Magnetically Driven Glass Spinner Flasks

### Screw Caps for 4500 and 4502 Series Flasks (Figure 8)

The 70 mm, 100 mm, and 120 mm screw cap assemblies are comprised of 3 components:

- ▶ Screw Cap (Figure 8C)
- ▶ Silicone Liner (Figure 8C)
- ▶ Shaft Retaining Nut (Figure 8A)

**NOTE:** Impeller assemblies provided with glass spinner flasks to be magnetically driven use shafts in 2 different diameters:

- 6 mm (1/4") diameter shafts made of polysulfone for flask sizes 125 mL to 1L.
- 8 mm (5/16") diameter shafts made of glass for flask sizes 3L and larger.

The center cap assembly provided with the specific capacity glass spinner flask includes a Shaft Retaining Nut of the correct size to work correctly with the diameter of shaft included in the impeller assembly.

When multiple sizes of glass spinner flasks are being disassembled and reassembled in the same working area. **NOTE:** It is important to not mix Shaft Retaining Nuts of different sizes.

An 8 mm diameter shaft will not fit through the center hole of a 6 mm Shaft Retaining Nut. A 6 mm diameter shaft will fit through the center hole of an 8 mm Shaft Retaining Nut, however, there will be excessive side-to-side wobble of the shaft and poor agitation.

#### Step 1 (4500, 4502, 4510, 4512 Series)

Secure the Silicone Liner into the top of the Screw Cap. To help secure the Silicone Liner, the Integrated Compression Fitting and outer edge of the Screw Cap have been undercut to create a "notch" into which the liner fits.

#### Step 2 (Figure 8A)

Remove the Shaft Retaining Nut from the cap. Slide the knurled, larger end of the Shaft Retaining Nut onto the Impeller Shaft on the Assembled Paddle.

#### Step 3 (Figure 8B)

Insert the PTFE Split Ring back onto the notch of the shaft from which it was previously removed during the Paddle Assembly process.

#### Step 4 (Figure 8C)

Insert the Completed Paddle Assembly and Retaining Shaft into the Screw Cap and tighten the assembly by turning the Shaft Retaining Nut clockwise until the nut is tightened down on the compression fitting. The paddle shaft should not be able to be withdrawn from the compression fitting. The Paddle Assembly should spin freely on the shaft if pushed in a circular motion in any direction.



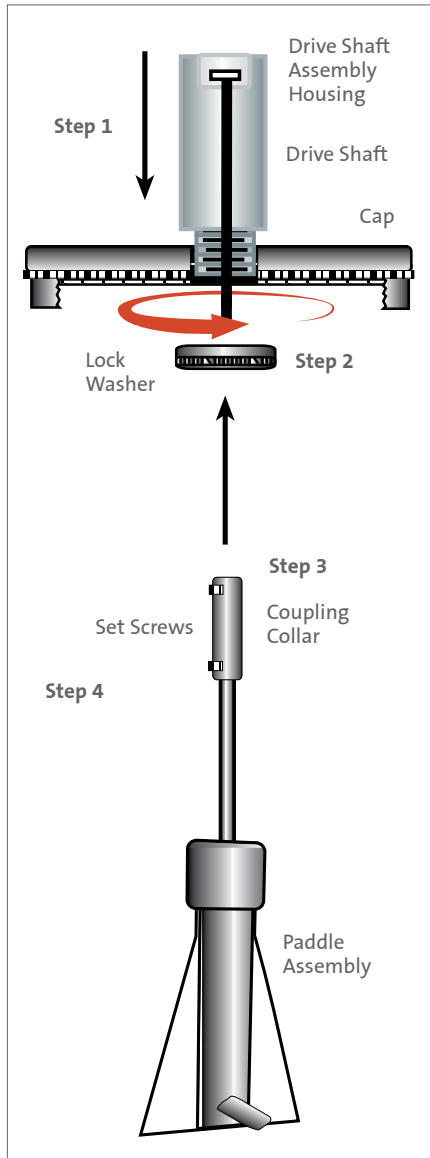


Figure 9. Direct drive cap assembly

## Center Cap Assemblies for Direct Drive Glass Spinner Flasks

### Connection of Direct Drive Systems with 100 mm or 120 mm Cap Assembly (Figure 9)

The use of Direct Drive with a Corning Glass Spinner Flask requires the separate purchase of one of the following two center cap assemblies, according to the center neck diameter of the flask.

- ▶ For flasks with 100 mm center opening (Corning Cat. No. 402614)

- ▶ For flasks with 120 mm center opening (Corning Cat. No. 400649)

The following steps describe how to assemble a direct drive center cap and how to attach the cap with the direct drive impeller assembly.

#### Step 1

Insert the Drive Shaft Assembly Housing through the center hole in the cap. The drive shaft should protrude out of the bottom of the cap assembly.

#### Step 2

Tighten the Lock Washer onto the threads of the Drive Shaft Assembly Housing under the cap.

#### Step 3

Slide the assembled Coupling Collar/Paddle Assembly up onto the protruding drive shaft.

#### Step 4

Tighten the Set Screws on both sides of the Coupling Collar so that the collar does not turn on the Drive Shaft.

Alternatively, slide the smaller opening on the Coupling Collar over the Drive Shaft in the Cap Assembly (Figure 10), and tighten the Upper Set Screw (Figure 11). Insert the stainless steel paddle Shaft into the larger opening in the bottom of the Coupling Collar (Figure 12) and then tighten the lower Set Screw on the Coupling Collar (Figure 13).

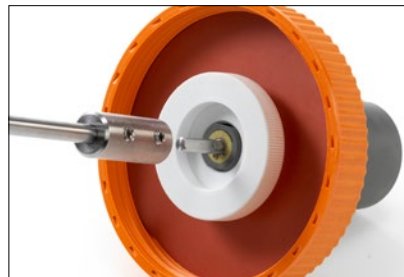


Figure 10. Insert drive shaft in cap assembly into the smaller opening of the coupling collar.



Figure 11. Tighten the upper set screw.

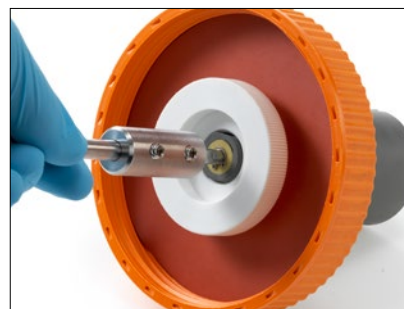


Figure 12. Insert paddle shaft into coupling collar.



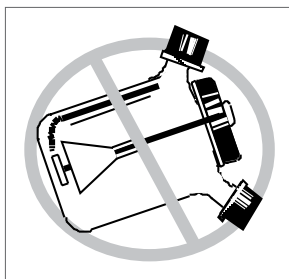
Figure 13. Tighten lower set screw on the coupling collar.

## Final Assembly of Vessels and Autoclaving

### Vessel Assembly

1. Insert the combined paddle and cap assembly into the vessel. It may be necessary on the larger vessels to bend the paddle blades slightly while the paddle assembly is being slid into the center neck of the vessel
2. If the blades should pop free from the blade retaining ring, lift the assembly up in the vessel and gently reinsert the blades back into the notches in the blade retaining ring.
3. Secure the screw cap onto the vessel by tightening it slightly on the threads of the center neck.  
**Important:** Do not tighten any of the cap assemblies (Center neck or Sidearm) on the vessel. Overtightening or securing the cap too tightly in or on the vessel can result in implosion of the vessel during autoclaving and/or fracturing of the center neck opening during autoclaving.
4. Loosely place the caps onto the sidearm ports. A slight clockwise turn of the cap onto the glass is sufficient to keep the caps from falling off the vessel while transporting it to the autoclave.
5. The vessel is now ready for autoclaving.

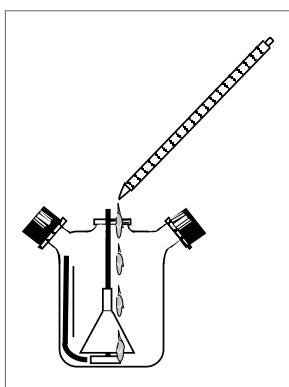
**NOTE:** Use of Vented Membrane Cap (Corning Cat. No. 1395-45LTMC) is recommended, as it will allow gas to exchange more readily through the sidearms.



**Figure 14.** Place spinner flask in an upright position when autoclaving the vessel. Do not lay on its side.

### Autoclaving

1. After assembly, place the vessel into an autoclave. We recommend that the vessels are autoclaved in an upright position. (Figure 14).  
**NOTE:** A small amount of deionized water may be added to the vessel to generate additional steam during autoclaving (Figure 15).
2. Check once more that the sidearm caps are still loosely attached, and that the cap on the center neck has not shifted during transport to the autoclave to allow for adequate transfer of steam into the vessel and venting during and after autoclaving.
3. If the vessel is too large to autoclave in an upright position, the cap and paddle assembly must be removed, autoclaved separately in an autoclave bag, and then aseptically reassembled in sterile conditions under a hood. Failure to do so may result in the bearing holder warping which will result in poor agitation of the paddle assembly.
4. Autoclave the vessel using a “liquid” cycle for a minimum of 30 minutes at 121° C and 15 psi. For larger vessels extended autoclaving may be necessary. All parts of the vessel have been designed to be autoclaved repeatedly using the conditions described above.
5. Allow the vessel to cool after autoclaving.



**Figure 15.** A small amount of water (5 to 10 mL of distilled or deionized water per 10L of vessel volume) can be added into the larger vessel to generate additional steam in the vessel during autoclaving.

# Basic Operation

## Filling the Vessel with Medium

This section provides basic information for cell culture methods using Corning Glass Spinner Flasks.

### Filling

Filling the vessel can be accomplished in a variety of ways, and is best done in a sterile biological hood or a clean room.

Small vessels (Center Neck or Sidearm Introduction):

1. Open the vessel after it cools by loosening the center cap assembly and gently shifting the cap and paddle slightly over, allowing it to rest on the lip of the center neck opening, or remove sidearm cap and rest in hood.
2. Introduce medium by pouring from pre-sterilized bottles or bags containing medium by gravity-feed or peristaltic pumps (Figure 16).
3. “In-line” addition is an alternative. Unsterilized medium can be added by filtering “in-line,” using a length of sterilized tubing connected to a 0.2  $\mu\text{m}$  filter attached between the unsterilized medium source and the vessel. Either gravity-feed or a peristaltic pump can be used to move the fluid. By using a filling bell supported by a ring stand (Figure 17), several vessels can be rapidly filled.

Large vessels (Sidearm Introduction):

1. Medium addition is usually done using the “in-line” sterilization method described above.
2. With larger flasks, medium addition is usually done via the sidearms rather than the center neck. Again, the use of a filling bell supported by a ring stand greatly simplifies this operation.

#### IMPORTANT NOTE:

We strongly recommend that a sterility check be accomplished by incubating the vessel containing the medium for 24 hours prior to inoculation of the vessel. This will insure that the sterilization and medium transfer were accomplished aseptically.

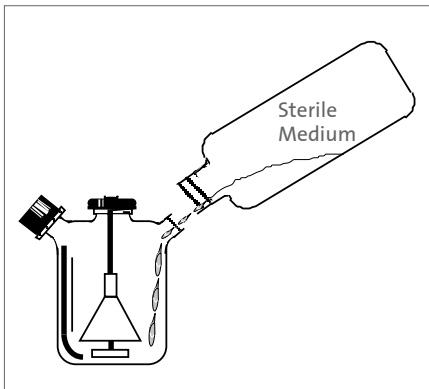


Figure 16. Small vessel being filled through a sidearm

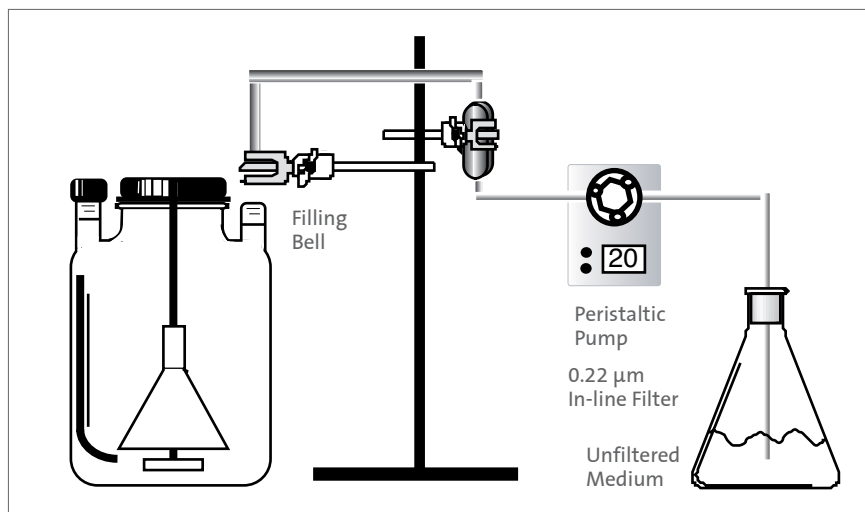


Figure 17. Large vessel being filled with the use of a filling bell and ring stand

## Inoculating and Stirring the Vessel

### Inoculating

The vessel should be inoculated in a sterile biological hood or in a clean room.

After the vessel passes the sterility check, cells can then be harvested and safely added to the vessel. Cells can be introduced by reconstituting cell pellets in a smaller volume of medium after harvesting. Adding cells is usually done using a syringe or length of sterile tubing into the center neck opening or sidearms. Inoculation densities are usually determined empirically using smaller test vessels such as T-flasks or small glass spinner flasks.

For cells which are anchorage-dependent, sterile microcarrier beads can be added to the vessel prior to or during the inoculation of the vessel. Cells and beads are often agitated intermittently during the attachment phase of the cells to the beads.

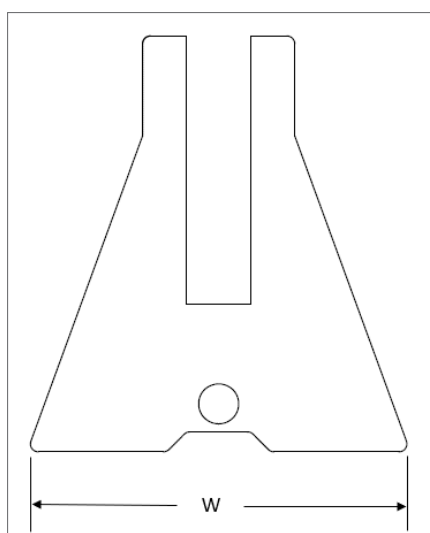
### Stirring

The Corning Glass Spinner Flasks have been engineered to achieve excellent mixing of vessel contents using slow speeds. The combination of the paddle design and vessel baffles provide optimal uplift. The surface-breaking design of the paddle also ensures adequate aeration of the cell culture medium at slower speeds. Higher speed stirring can be detrimental and is best done by slowly adapting cells to the higher shear forces which often develop at the higher speeds.

Initial stirring speeds for flasks of various flask sizes are listed below. These speeds should be regarded as suggestions only. Final stirring speed needs to be determined empirically.

Flask Size	RPM
100 mL to 1,000 mL	50 - 60
3,000 mL to 8,000 mL	40 - 50
15,000 mL to 36,000 mL	30 - 40

**NOTE:** It is recommended when scaling up to a larger flask to begin by using a stirring speed that results in approximately the same distance (circumference) traveled by the impeller blade tip per minute.



Flasks Size	Impeller Blade Width (W) (mm)	Circumference Traveled per Revolution (mm)
125 mL	40	126
250 mL	45	141
500 mL	59	185
1L	79	248
3L	121	380
6L	149	468
8L	151	474
15L	149	468
36L	172	540

## Stirring by Direct Drive Motor, Attaching Motor to Cap Assembly

### Step 1 (Figures 19 and 20)

Note the location of the two engagement slots for the bayonet fittings (see Figure 18, Dowel Pins, 1/8" x 1") on the top of the drive shaft (Figure 19). The dowel pins on the bottom of the Direct Drive Motor (Figure 20) will slide into these slots to secure the motor in place for operation.

### Step 2 (Figure 21)

Tilt the motor slightly from vertical and slide the yoke coupling into the cavity on the top of the direct drive shaft (Figure 21). The yoke dowel pins will engage with the drive pin inside of the cavity when the motor is correctly oriented.

### Step 3 (Figure 22)

Turn the motor until the dowel pins on the motor drive adapter assembly line up with the vertical section of the bayonet fitting. You can use the end of the dowel pins that are exposed on the motor drive adapter to help you orient the inside portion of the dowel pins with the bayonet fitting as shown in Figure 22. You will know that the dowel pins are correctly oriented when the motor unit drops slightly into the vertical slots.

### Step 4 (Figure 18)

When the dowel pins have dropped into the slots, turn the motor slightly clockwise to lock the entire assembly together.

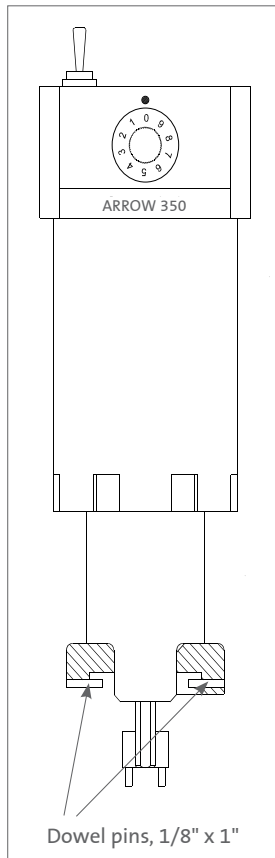


Figure 18. Direct Drive Motor

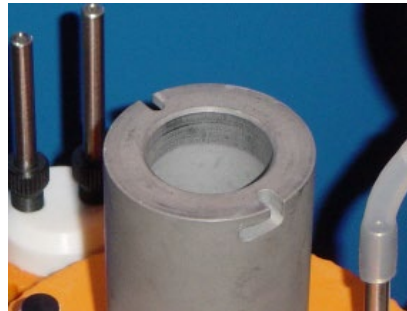


Figure 19. Engagement slots for bayonet fittings

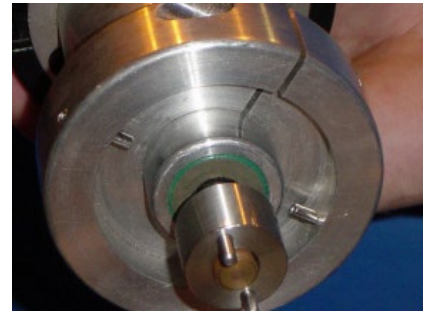


Figure 20. Closeup of bayonet fittings and yoke dowel pins



Figure 21. Coupling of motor and direct drive shaft



Figure 22. Correct orientation of motor on drive shaft

## Disassembly, Care, and Maintenance

### Disassembly

Remove the center cap and impeller assembly from the vessel.

Unscrew the shaft retaining nut to separate the cap assembly and the impeller assembly.

**CAUTION:** It is possible for a glass impeller shaft to become seized inside the compression fitting detail of the cap. If this is encountered, place the cap with impeller assembly into an autoclave and heat for a few minutes to soften the plastic components. This should allow the shaft to be removed from the cap without breaking the shaft. For additional precaution against cuts if the shaft does break during the attempt to remove, place a protective layer of material between shaft and hand.

Remove the split ring from the top of the impeller shaft to separate impeller assembly from the shaft retaining nut.

Follow the initial assembly directions in reverse to finish disassembling the impeller assembly and the cap assembly.

**NOTE:** We recommend placing the parts into a dilute cleaning solution to soak as soon as possible after disassembly. If the flask needs to be decontaminated, pour out the contents into a separate vessel (kill tank), and fill the flask (including the internal components) as soon as possible with a bleach solution. After treatment, rinse thoroughly and wash or soak in the cleaning solution as above.

### Care and Maintenance

The Corning Glass Spinner Flask products use materials supporting multiple standard autoclaving cycles typical with long-term product use. Extended autoclaving times at elevated temperatures, or soaking the vessel or parts in acids, bases, or strong oxidizing agents for unusually long lengths of time will result in these materials deteriorating more quickly than usual.

Plastic components, particularly those made using PTFE sheet material, may warp during autoclaving. This is normal but may eventually result in the impeller assembly not spinning freely. If this occurs, it will be necessary to replace the impeller assembly.

**CAUTION:** Avoid the use of organic solvents on any of the plastic or synthetic rubber parts. They may weaken and significantly reduce the life span of the parts.

### Basic Care

Care should be taken when washing the glass vessels to minimize bumping against other glassware or the sides of sinks. This could cause damage to the glass which could result in glass breakage during a subsequent autoclaving cycle.

Over time, buildup of biological or mineral residues on the walls of the glass vessels or parts may occur. If this happens, consult the section entitled "Rigorous Glassware Cleaning Protocols for Severely Soiled Flasks".

### Rigorous Glassware Cleaning Protocols for Severely Soiled Flasks

After extended use, residues may build up on the flasks and components requiring more rigorous cleaning of the flasks. These residues may include pyrogenic materials, buildup of dead cells and debris on the vessel, and components of the cell culture medium. To minimize the buildup of these materials, we recommend that the parts are soaked in a dilute cleaning solution as soon as cell culture is terminated.

Disassemble the vessels to facilitate penetration of the cleaning agent. Several protocols are described below. After these treatments have been performed, be sure to wash all components again as was described in the "Standard Glassware Cleaning Protocol". Glassware cleaning is reasonably straightforward. Silicone and synthetic rubber components tend to absorb cleaners and other chemicals, so more caution must be taken to minimize contact with them.



## Depyrogenation

### 1. Baking

Glassware can be baked for at least 12 hours at 120°C (250°F), then clean as described in the “Standard Glassware Cleaning Protocol.”



**DO NOT USE HEAT TREATMENT ON ANY OF THE PLASTIC OR SILICONE/SYNTHETIC RUBBER COMPONENTS.**

### 2. Chemical Treatment

Soak flask components in solutions of sodium hydroxide (0.1 N to 1.0 N NaOH) for at least 1 hour, followed by soaking and then repeated washing with distilled or deionized water. To ensure that all traces of NaOH are gone, check the pH of the soaking solutions to ensure that they are not alkaline. Some investigators chase the NaOH treatment with a weak solution (0.1 N to 1.0 N) of phosphoric acid to neutralize the NaOH prior to rinsing in distilled water.

## Removal of Biological and Organic Residues

### 1. Ashing

Glassware can be treated by baking at a temperature of at least 840°F (450°C) for at least 3 hours. This removes all organic compounds to a mineral and carbon ash.



**DO NOT USE THIS TREATMENT ON ANY OF THE PLASTIC OR SILICONE/SYNTHETIC RUBBER COMPONENTS.**

### 2. Chemical Treatment with NaOH

Follow the procedure described above under “Depyrogenation.”

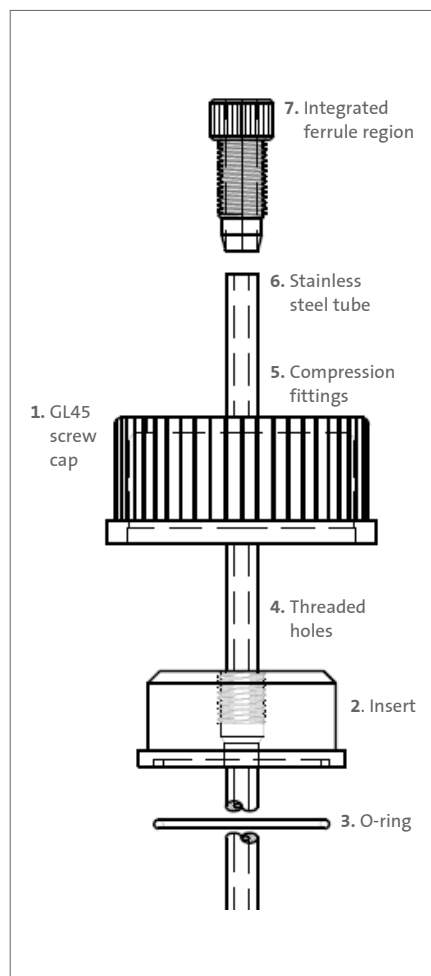
### 3. Chemical Treatment with Chromic Acid

This treatment is very effective in decomposing biological molecules, and is helpful to reduce mineral deposits. Be sure to follow all normal safety precautions when handling acid solutions. Chromic acid is made by dissolving 20 g of Na<sup>+</sup> or K<sup>+</sup> chromate in sufficient water to make a paste. Add 300 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. Glassware can be treated for as long as 24 hours. Rinse and clean afterwards as described in the “Standard Glassware Cleaning Protocol.”

## Removal of Mineral Deposits

Residual scale which is built up on glassware may be removed to varying extents by treatments with acids, such as sulfuric, nitric, hydrochloric acid, or chromic acids as described above. The nitric acid treatment is especially useful in removing buildup of material causing corrosion of stainless steel parts. Follow safety procedures in handling as with chromic acid above. Start with short, mild treatments and gradually increase the strength of the reagent and length of exposure as necessary.

# Using Sidearm Fittings for Augmented Operations



## Optional Operation

### Sidearm Fittings

Corning provides unique opportunity for achieving better performance from your glass spinner flasks through use of accessory sidearm fittings, available for both angled sidearm flasks and vertical sidearm flasks.

Combined with the use of system controllers, flasks with accessory sidearm fittings can be used to:

- Supply or control additional gases (i.e., oxygen) to the flask to improve the exchange of the gas with the liquid medium
- Add and/or remove fresh medium in fed-batch operations to replenish spent nutrients in the medium
- Perform in-line filtration of the culture medium using microporous filters to remove spent medium which may inhibit cell growth
- Add sensors and liquid addition ports into the vessel to help monitor and/or control pH, O<sub>2</sub> or temperature
- Inoculate the cell culture medium at the beginning or during a cell culture process

### Basic details of sidearm fittings

All sidearm fittings are based on use of the GL45 glass thread on the sidearm and a GL45 screwcap.

The GL45 screw cap (1) is made of polypropylene. The center of the cap is open to accept an insert.

The insert (2) is made of PET. An O-ring (3) is located on the bottom surface of the insert to create a seal with the opening of the glass sidearm. The upper surface of the insert contains the threaded holes (4) necessary to accept the compression fittings and tubes corresponding to the configuration of the specific sidearm fitting.

The compression fittings (5) are made of PPO. The stainless steel tube (6) passes through the hollow center of the compression fitting. An integrated ferrule region (7) exists on the tip of the compression fitting. When the compression fitting is screwed finger-tight into the insert, the ferrule region compresses onto the outside diameter of the stainless steel tube. This compression creates a seal with the tube and holds the tube in position. The length of tubing extending into the flask can be adjusted by loosening the compression fitting, sliding the tubing to the desired location, and then retightening the compression fitting.

**NOTE:** The degree of compression to seal and secure the tubing is achieved with only a modest, “finger-tight”, twist of the compression fitting. Screwing the compression fitting in at higher torque will damage the ferrule region, making the compression fitting non-functional.

Tubes for sidearm fittings are made of grade 316 stainless steel. Most sidearm fittings use tubes of ¼ inch outside diameter or 1/8 inch outside diameter or a combination of these two sizes. There are also sidearms for larger volume media handling which use ½ inch outside diameter tubing.

Sidearm fittings for flasks with vertical sidearms use straight tubing. As such, they can be adjusted vertically for desired depth of penetration into the flask contents, with any excess tubing length residing above the sidearm cap.

The tubing used in sidearm fittings for flasks with angled sidearms is bent. There is only a small degree of location adjustment possible with sidearm fittings of this type.

Sidearm fittings can be autoclaved.



Cat. No. 1395-45LTMC

### Using sidearms for venting the flask

Any sidearm on a flask can be closed off using the screw cap originally provided with the flask. This standard GL45 screw cap is Cat. No. 1395-45LTC.

Flask venting can be accomplished using either of the follow two products available from Corning:

Cat. No. 1395-45LTMC: This is a standard GL45 screw cap, however it has a 3 mm hole in the center of the cap. Beneath the hole is a permanently attached 0.2 µm PTFE filter membrane. The membrane diameter is 25 mm. This cap is generally suitable for operations using a 1/8" inlet gassing fitting.

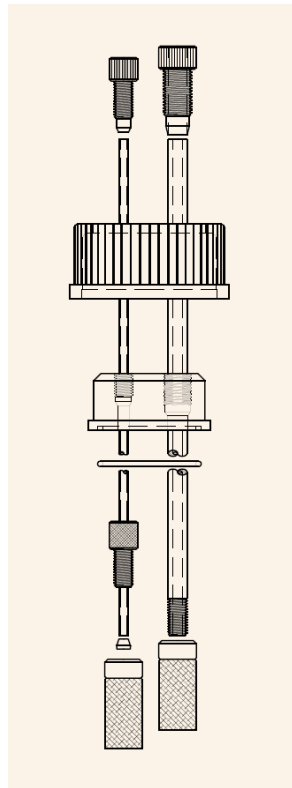
Cat. No. 4519-106: This product is built like a typical sidearm fitting. The Insert of the fitting is threaded to accept 50 mm replaceable 0.2 µm PTFE filters with 1/8" NPT threads. One filter is provided with the product. This product is generally suitable for operations using a 1/4" inlet gassing fitting.

### Selecting Fittings for Vertical Sidearm Flasks

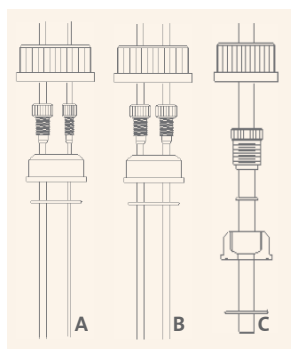
#### Fittings for inputting Gas

The image below represents the fitting (Cat. No. 4519-104) which uses both a 1/8" OD tube (left) and a 1/4" OD tube (right). Single tube versions (Cat. Nos. 4519-100 and 4519-102) using 1/8" OD tubing and 1/4" OD tubing, respectively. All three products are produced with the same length tubing and are intended for use in all three sizes of flasks with vertical sidearms, 8L, 15L, and 36L.

Description	Cat. No.		
	8L	15L	36L
Single 1/8" OD tube with filter	4519-100	4519-100	4519-100
Single 1/4" OD tube with filter	4519-102	4519-102	4519-102
One 1/8" OD tube with filter and One 1/4" OD tube with filter	4519-104	4519-104	4519-104



Gas fitting (Cat. No. 4519-104)



Media handling fittings

### Fittings for Media Handling

The image reflects the variations in media handling fittings available for flasks with vertical sidearms. The image to the left of the collection (A) has two tubes, one with 1/8" OD and the other with 1/4" OD. The image in the center (B) has two tubes, both with 1/4" OD. The image to the right (C) has one tube of 1/2" OD.

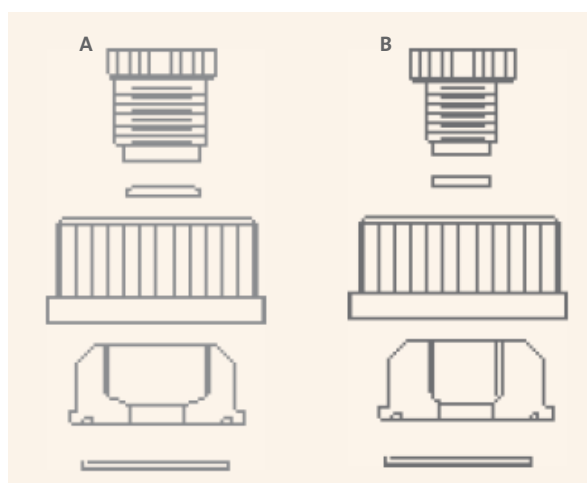
Products identified for use with the 36L flask will also work in 8L and 15L flasks with the excess tubing length protruding above the glass spinner flask sidearm. For convenience, the tubing length used in products identified for use with 8L and 15L flasks is 3 inches shorter than the tubing used in the same products for 36L flasks.

Description	Cat. No.		
	8L	15L	36L
Two tubes, both 1/8" OD (B)	4519-112	4519-112	4519-114
Two tubes, both 1/4" OD (B)	4519-116	4519-116	4519-118
Two tubes, one 1/8" OD, one 1/4" OD (A)	4519-120	4519-120	4519-122
One tube, 1/2" OD (C)	4519-124	4519-124	4519-126

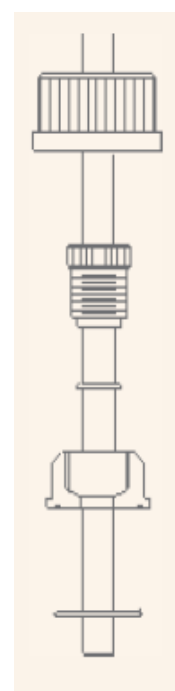
### Fittings for Insertion Probes

Corning offers two products which can be used to secure a pH, O<sub>2</sub>, or temperature probe in the vertical position within a vertical sidearm flask. For probes with a 12 mm (1/2") diameter (Cat. No. 4519-110) and for probes with an 18 mm (3/4") diameter (Cat. No. 4519-172) is recommended. The probe chosen for use has to have a body that is completely cylindrical in order to pass through the holding nut of the fitting. The holding nut provided with Cat. No. 4519-110 has an inside diameter of 13 mm. The holding nut provided with Cat. No. 4519-172 has an inside diameter of 19 mm. The O-ring provided fits around the diameter of the probe and is compressed when the holding nut is screwed into the insert to hold the probe in position.

Cat. No.	Description
4519-110	For pH, O <sub>2</sub> , or temperature probes with 12 mm outside diameter body
4519-172	For pH, O <sub>2</sub> , or temperature probes with 18 mm outside diameter body



Cat. Nos. 4519-172 (A) and 4519-110 (B)



A 1/2" OD probe fitted into Cat. No. 4519-110.

## Selecting Fittings for Flasks with Angled Sidearms

Fittings for angled sidearm flasks are provided in three different styles: Dual fittings, combination fittings, and combination triple fittings.

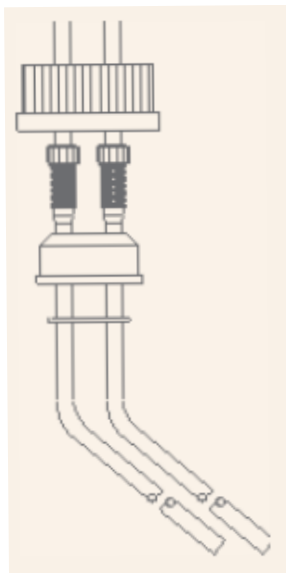
Dual Fittings include two tubes which are both bent so that once past the angled sidearm, the tubes terminate vertically, are below the liquid level in the flask, in the region between the impeller assembly and the side wall of the flask.

Dual fittings are provided with:

- Both tubes with 1/8" OD
- Both tubes with 1/4" OD
- One tube with 1/8" OD and the other tube with 1/4" OD

### Dual fittings, both tubes bent

Description	Cat. No.						
	500 mL	1L	3L	6L	8L	15L	36L
Both tubes 1/8"	--	4519-150	4519-151	4519-152	4519-153	--	--
Both tubes 1/4"	--	4519-154	4519-155	4519-156	4519-157	4519-170	4519-170
One tube 1/8", One tube 1/4"	4519-174	4519-173	--	--	4519-121	--	--



Representation of a Dual fitting with both tubes 1/4" OD.

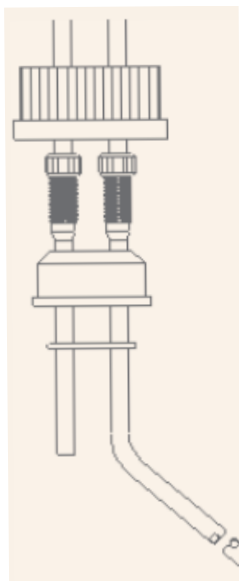
**Combination Fittings** include one tube which is bent and another shorter tube which is straight. The straight tube is approximately 6 inches long and is intended to be located so that its open end is above the level of liquid in the flask.

Combination fittings are provided with:

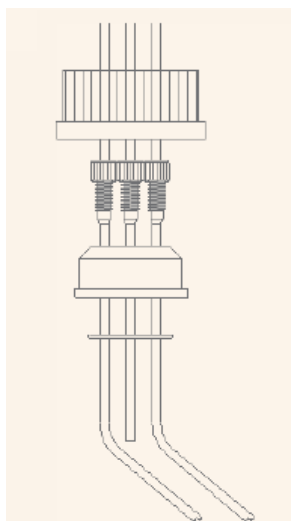
- Both tubes with 1/8" OD
- Both tubes with 1/8" OD

Combination fittings, one tube bent, one tube straight (6 inches overall length)

Description	Cat. No.						
	500 mL	1 L	3 L	6L	8L	15L	36L
Both tubes 1/8"	--	4519-158	4519-159	4519-160	4519-161	--	--
Both tubes 1/4"	--	4519-162	4519-163	4519-164	4519-165	4519-171	4519-171



Representation of a Combo fitting with both tubes 1/4" OD.



Representation of a Combo triple fitting.

Combination Triple Fittings include three tubes for a combined functionality of the dual and combo fittings. Two tubes are bent to terminate below the liquid level in the flask. The third tube is short and straight to terminate above the liquid level in the flask.

Combination triple fittings are provided with all three tubes 1/8" OD.

Combination triple fittings, two tubes bent, one tube straight (6 inches overall length)

Description	Cat. No.						
	500 mL	1L	3L	6L	8L	15L	36L
All tubes 1/8"	--	4519-166	4519-167	4519-168	4519-169	--	--

### Using the Center Neck Cap for Accessory Functions

It is possible to use the center cap of glass spinner flasks as an area to place input and output tubing and to mount insertion type pH, O<sub>2</sub>, or temperature probes.

For flasks with 120 mm center necks that are driven magnetically, Corning provides three different cap assemblies with four additional fittings installed as represented in the following image (Figure 23).

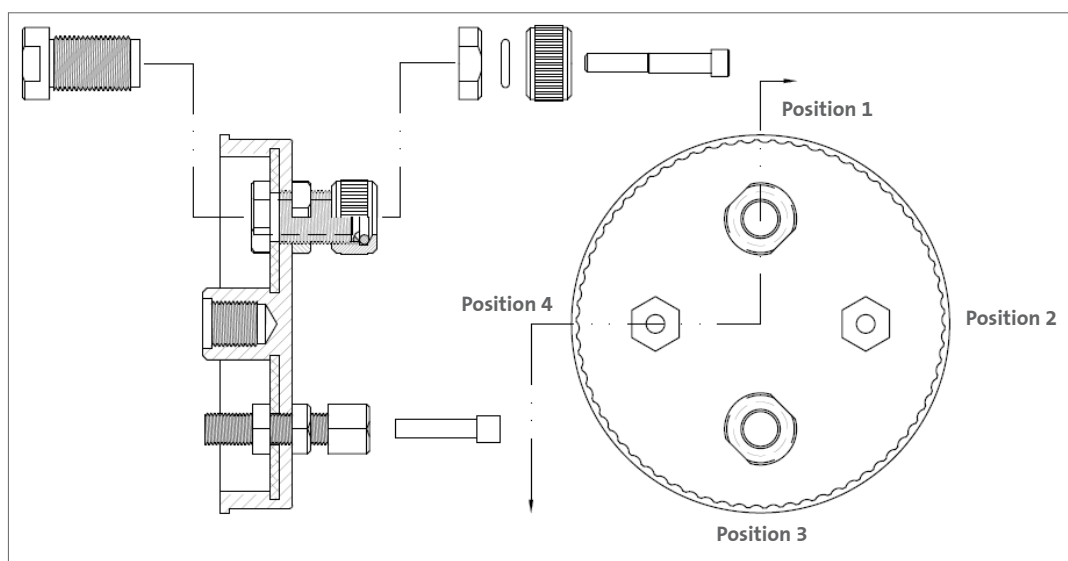


Figure 23.

Cat. No.	Position 1	Position 2	Position 3	Position 4
402576	12 mm OD probe	1/4" OD tubing	12 mm OD probe	1/4" OD tubing
402577	12 mm OD probe	1/4" OD tubing	12 mm OD probe	3/8" OD tubing
402579	3/8" OD tubing	3/8" OD tubing	3/8" OD tubing	1/4" OD tubing

The products are provided with blocking pins in each of the four fittings.

All fittings are compression type to secure around the OD of the probe or tubing.

The three center cap assemblies listed are supplied without an Impeller Attachment Nut. The following product must be purchased separately to attach an impeller assembly to the cap assembly.

Cat. No.	Description
BP-3413-9042	Nut assembly for attaching center cap to magnetically driven impellers using 5/16" (8 mm) OD shaft, includes O-ring



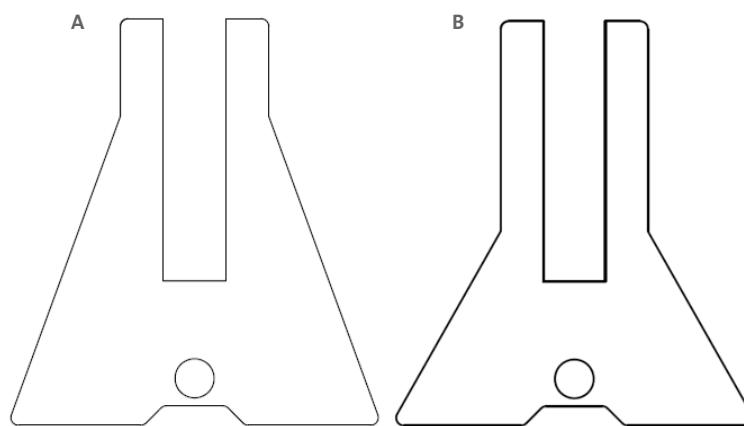
Use of one of the three center caps with additional fittings typically requires use of an impeller assembly where the blade of the impeller assembly has been modified (reduced in size) to provide room for the probe to extend through the cap and into the liquid contained in the vessel.

The following are products for magnetically driven impeller assemblies that have been modified for use with probes.

Description	Cat. No.				
	3L	6L	8L	15L	36L
Impeller assembly modified for use with probes	402648	401577	401392	401661	401901

Impeller assemblies modified for use with probes are provided with a 5/16" (8 mm) OD shaft made of stainless steel instead of glass.

The following is a diagram of how the blade shape is modified. (A) Standard shape impeller blade for a 6L product; (B) 6L impeller modified for use with probes.



### Glass Spinner Flask Component Materials

Component	Material Description
Flask	Corning 7740 Type I, Class A borosilicate glass
Sidearm Screw Cap	PP
Sidearm Pouring Ring	PP
<b>Center Cap Assembly</b>	
Cap	PBT 30% glass filled
Liner	Silicone
Impeller Shaft Retaining Nut	Polyetherimide
Retaining Nut O-ring	Fluoroelastomer
<b>Impeller Assembly</b>	
Bearing Holder	PTFE
Impeller Blade	PTFE
Magnet	PTFE coated
Blade Retaining Sleeve	PTFE
Shaft Retaining Washer	PTFE
Shaft, Plastic	Polysulfone
Shaft, Steel	316 stainless steel
Shaft, Glass	Corning 7740 Type I, Class A borosilicate glass
<b>Sidearm Fittings</b>	
Screw Cap	PP
Insert	PET
Insert O-ring	Fluoroelastomer
Holding Nut	PPO
Tubes	316 stainless steel
C-clip	316 stainless steel

PP: Polypropylene; PBT: Polybutylene Terephthalate; PTFE: Polytetrafluorethylene; PET: Polyethylene Terephthalate; PPO: Polyphenylene Oxide

The components of Corning glass spinner flasks identified above can be autoclaved and are chemically compatible with acidic (1 N HCl) and basic (1 N NaOH) cleaning solutions.

# Ordering Information

## Accessories and Spare/Replacement Parts for Corning Glass Spinner Flasks

### Glass Flasks

The following products are established to enable purchasing the glass flask only for each of the sizes of angled sidearm glass spinner flasks.

Cat. No.	Description
BP-3405-100	Glass flask only, 125 mL size glass spinner flask with angled sidearms and 70 mm center neck
BP-3405-102	Glass flask only, 250 mL size glass spinner flask with angled sidearms and 70 mm center neck
BP-3405-104	Glass flask only, 500 mL size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-106	Glass flask only, 1L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-108	Glass flask only, 3L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-110	Glass flask only, 6L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-112	Glass flask only, 8L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-114	Glass flask only, 15L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3405-116	Glass flask only, 36L size glass spinner flask with angled sidearms and 100 mm center neck
BP-3505-108	Glass flask only, 3L size glass spinner flask with angled sidearms and 120 mm center neck
BP-3505-110	Glass flask only, 6L size glass spinner flask with angled sidearms and 120 mm center neck
BP-3505-112	Glass flask only, 8L size glass spinner flask with angled sidearms and 120 mm center neck
BP-3505-114	Glass flask only, 15L size glass spinner flask with angled sidearms and 120 mm center neck
BP-3505-116	Glass flask only, 36L size glass spinner flask with angled sidearms and 120 mm center neck

The glass flasks for vertical sidearm glass spinner flasks are available by purchasing the original catalog number of the overall glass spinner flask product, as listed below.

Cat. No.	Description
4510-8L	Glass spinner flask, flask and caps only, 8L, 100 mm center neck, 4 GL45 vertical sidearms
4510-15L	Glass spinner flask, flask and caps only, 15L, 100 mm center neck, 4 GL45 vertical sidearms
4510-36L	Glass spinner flask, flask and caps only, 36L, 100 mm center neck, 6 GL45 vertical sidearms
4512-8L	Glass spinner flask, flask and caps only, 8L, 120 mm center neck, 4 GL45 vertical sidearms
4512-15L	Glass spinner flask, flask and caps only, 15L, 120 mm center neck, 4 GL45 vertical sidearms
4512-36L	Glass spinner flask, flask and caps only, 36L, 120 mm center neck, 6 GL45 vertical sidearms

## Impeller Assemblies

Impeller assemblies should be replaced as an entire assembly. The following products are established to enable purchasing entire impeller assemblies.

Cat. No.	Description
BP-3401-100	Impeller assembly, for 125 mL glass spinner flask, magnetically driven, 1/4" PS (plastic) shaft
BP-3401-102	Impeller assembly, for 250 mL glass spinner flask, magnetically driven, 1/4" PS (plastic) shaft
BP-3401-104	Impeller assembly, for 500 mL glass spinner flask, magnetically driven, 1/4" PS (plastic) shaft
BP-3401-106	Impeller assembly, for 1L glass spinner flask, magnetically driven, 1/4" PS (plastic) shaft
BP-3401-108	Impeller assembly, for 3L glass spinner flask, magnetically driven, 5/16" glass shaft
BP-3401-110	Impeller assembly, for 6L glass spinner flask, magnetically driven, 5/16" glass shaft
BP-3401-112	Impeller assembly, for 8L glass spinner flask, magnetically driven, 5/16" glass shaft
BP-3401-114	Impeller assembly, for 15L glass spinner flask, magnetically driven, 5/16" glass shaft
BP-3401-116	Impeller assembly, for 36L glass spinner flask, magnetically driven, 5/16" glass shaft

The following components of impeller assemblies which tend to be easy to break or lose are available for individual purchase.

Cat. No.	Description
BP-3411-030	PTFE split ring (ID x CS; 4.5 x 1.8 mm), for impellers with 1/4" (6.4 mm) OD shaft
BP-3411-031	PTFE split ring (ID x CS; 6 x 2.6 mm), for impellers with 5/16" (8 mm) OD shaft
BP-3411-090	Silicone plug (OD x Length; 9.5 x 4.8 mm), used in glass spinner flask impellers using a 1/4" shaft, typical in 1L size and smaller glass spinner flask
BP-3411-095	Silicone plug (OD x Length; 19 x 9.5 mm), used in glass spinner flask impellers using a 5/16" shaft, typical in 3L and larger glass spinner flask
BP-3411-170	Plastic shaft 1/4" OD (OD x Length; 6.4 x 84 mm), Polysulfone (PS), w/ PTFE split rings, for 125 mL and 250 mL size glass spinner flasks
BP-3411-172	Plastic shaft 1/4" OD (OD x Length; 6.4 x 122 mm), Polysulfone (PS), w/ PTFE split rings, for 500 mL size glass spinner flask
BP-3411-173	Plastic shaft 1/4" OD (OD x Length; 6.4 x 147 mm), Polysulfone (PS), w/ PTFE split rings, for 1L size glass spinner flask
BP-3411-174	Glass shaft 5/16" OD (OD x Length; 8 x 189 mm), with PTFE split ring, for 3L size glass spinner flask
BP-3411-175	Glass shaft 5/16" OD (OD x Length; 8 x 235 mm), with PTFE split ring, for 6L size glass spinner flask
BP-3411-176	Glass shaft 5/16" OD (OD x Length; 8 x 239 mm), with PTFE split ring, for 8L and 36L size glass spinner flask
BP-3411-177	Glass shaft 5/16" OD (OD x Length; 8 x 219 mm), with PTFE split ring, for 15L size glass spinner flask

If breakage of the glass shafts used in impeller assemblies become problematic, and the application can allow use of stainless steel impeller shafts, then the following products are available to enable purchase of a stainless steel impeller shaft

Cat. No.	Description
BP-3611-083	Stainless steel shaft 1/4" OD (OD x L; 6.4 x 148 mm), with PTFE split ring, for 1L size glass spinner flask
BP-3611-084	Stainless steel shaft 5/16" OD (OD x L; 8 x 186 mm) with PTFE split ring, for 3L size glass spinner flask
BP-3611-085	Stainless steel shaft 5/16" OD (OD x L; 8 x 232 mm) with PTFE split ring, for 6L size glass spinner flask
BP-3611-086	Stainless steel shaft 5/16" OD (OD x L; 8 x 235 mm) with PTFE split ring, for 8L and 36L size glass spinner flask
BP-3611-087	Stainless steel shaft 5/16" OD (OD x L; 8 x 216 mm) with PTFE split ring, for 15L size glass spinner flask

### Center Neck Cap Assemblies for Magnetically Driven Glass Spinner Flask

The following products are available to enable purchasing the center neck cap assembly for magnetically driven glass spinner flasks. The cap assembly as provided includes the nut assembly needed for attaching the impeller assembly to the cap.

Cat. No.	Description
BP-3402-100	Center cap assembly, for glass spinner flask with 70 mm center neck using impeller assembly with 1/4" OD shaft
BP-3403-100	Center cap assembly, for glass spinner flask with 100 mm center neck using impeller assembly with 1/4" OD shaft
BP-3404-100	Center cap assembly, for glass spinner flask with 100 mm center neck using impeller assembly with 5/16" OD shaft
BP-3502-099	Center cap assembly, for glass spinner flask with 120 mm center neck using impeller assembly with 5/16" OD shaft

The nut assemblies and the O-ring used for either of the two sizes of nut assemblies are available for individual purchase.

Cat. No.	Description
BP-3413-9041	Nut assembly for attaching center cap to magnetically driven impellers using 1/4" (6.4 mm) OD shaft, includes O-ring
BP-3413-9042	Nut assembly for attaching center cap to magnetically driven impellers using 5/16" (8 mm) OD shaft, includes O-ring
BP-3812-005	O-ring, for impeller nut, size #112 (ID x cross section (CS)); 12.4 x 2.6 mm

### Sidearm Fittings

Sidearm Fittings should be replaced as an entire assembly. The following components of sidearm fittings which tend to be easy to lose are available for individual purchase.

Cat. No.	Description
BP-3802-002	O-ring, for sidearms fittings, 1 1/4" ID x 1/16" cross section (CS), FKM
BP-3802-004	Nut, compression, for 1/8" OD tubes in sidearm fittings, PPO material with 1/4-28 thread
BP-3803-004	Nut, compression, for 1/4" OD tubes in sidearm fittings, PPO material with 3/8-24 thread

### Direct Drive Cap Assemblies

The following component used in direct drive cap assemblies Cat. Nos. 402614 (100 mm) and 400649 (120 mm) is available for individual purchase. The component fits either size of direct drive cap assembly.

Cat. No.	Description
BP-3921-005	Nut, drive attachment, material PTFE, for glass spinner flask direct drive cap assembly

# Frequently Asked Questions and Troubleshooting Guide

Use the following guide to help you with the occasional problem you may experience with your Corning® Glass Spinner Flasks. These commonly asked questions will guide you prior to contacting Corning Scientific Support.

## 1. The medium in my flask is turning alkaline prior to addition of cells.

The flask may have been improperly washed using a detergent not designed for cell culture. Rewash the flask and components using a detergent designed for cell culture and rinse thoroughly with distilled water.

## 2. The impeller in my magnetically driven flask is not turning smoothly.

The flask may not be centered above the magnet inside the magnetic stirrer. Try moving the flask slightly on the magnetic stirrer until smooth motion is observed.

## 3. The impeller in my magnetically driven flask turns smoothly at slow speeds, but begins to turn in a disruptive or intermittent manner as the speed of the magnetic stirrer is increased.

The speed of the magnetic stirrer is set too high or has been increased too quickly. As a result, magnetic decoupling is occurring or the viscosity of the medium is too high, also resulting in decoupling.

## 4. What volume should I place in the flask?

### ▶ For New Users:

It is good to start at 50% of the working volume of the flask. In subsequent runs, add 10% more volume each time until cell density plateaus or maximum working volume of flasks is achieved.

### ▶ For Users of Conventional Flasks:

Start at the same volume as your current flask. In subsequent runs, add 10% more volume each time until cell density plateaus or maximum working volume of flasks is achieved.

**NOTE:** If the cell density plateaus before the maximum working volume is achieved, you may need to use an augmented operation to boost productivity.

## 5. What is the correct speed to spin a flask and how do I adjust the speed as my flask size changes?

First, look at the recommended guidelines for each flask size (see Section Stirring).

### ▶ For New Users:

In small flasks, keep the tip speed between 3 to 5 in/sec. In large flasks, keep tip speed between 10 to 15 in/sec. In subsequent runs, try and increase flask tip speed until cell density plateaus, or cell viability drops.

### ▶ For Users of Conventional Flasks:

Start at the same speed as your current flask. In subsequent runs, try and increase the flask tip speed until cell density plateaus, or cell viability drops.

## 6. Why should I use Direct Drives and what size?

The decision to choose a Direct Drive is mainly due to magnetic decoupling occurring at greater than 8L sizes, especially at the 15L and 36L sizes. Magnets in stirrer plates are too small and do not have enough power to stir large paddles and the increased volumes of liquid that are in the larger flasks. Direct Drives couple the drive shaft and motor together to aid in better stirring.

## 7. Why choose vertical sidearms rather than angled sidearms?

The choice of whether to use an angled or vertical sidearm is mainly based on versatility and ease of operation. It is easier to operate flasks and to position glass and stainless steel sensor probes into the medium of flasks with vertical sidearms than those with angled sidearms. Introducing gases into a flask with a tube positioned in a vertical orientation is easier than bending a tube through an angled sidearm configuration. In addition, when side arms are in a vertical orientation, more sidearms can be added and thus more fittings can be utilized in a vertical orientation creating more options for the end user.

**8. Why choose an augmented and/or bioreactor process?**

Augmenting your spinner flask for headspace overlays, direct sparging, media transfer, and/or pH and oxygen monitoring is mainly considered in order to increase cell density and productivity in the larger flasks. As cell culture volume increases, a decrease in cell density may occur due to factors such as nutrients and oxygen becoming limited in the flasks. While Corning® spinner flasks are better than conventional flasks in dealing with these issues, additional operations are still needed to help overcome these limitations and boost productivity.

**9. What type of augmented operation should I use?**

There are a number of easy solutions to some of these limitations. A Corning Application Specialist can help you in configuring your flasks for these operations. The options available are detailed in the Ordering Information section for sidearm fittings.



For more specific information on claims, visit [www.corning.com/certificates](http://www.corning.com/certificates).

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