

# Hypoxia Incubator Chamber



**Chamber for generation of a hypoxic environment for tissue culture**

Catalog # 27310

1 Chamber

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## Product Description

The Hypoxia Incubator Chamber is a self-contained and sealed chamber that fits inside existing laboratory incubators. Each unit utilizes a surface-type seal in which all portions of the O-ring are uniformly compressed by a ring clamp for a reliable, air-tight seal. The cylindrical walls and semi-spherical top and bottom minimize gas flow resistance and eliminate dead space during initial purging. The chambers have an integrated stacking feature for storage during or after experimentation. All units are molded from high quality polycarbonate and will not break, crack or cloud with extended use. Capacity: 84 x 35 mm plates, 27 x 60 mm plates, 12 x 100 mm plates, 12 x 96-well plates, 18 x 25 cm<sup>2</sup> flasks.

## Properties

**Storage:** Store at 15 - 25°C.

**Shelf Life:** Not applicable.

**Contains:**

- Lid\* (Polycarbonate)
- Base\* (Polycarbonate) with O-ring and Tubing Clamp
- Tray\* (Polycarbonate)
- Ring Clamp
- O-ring
- Tubing Clamps for Hypoxia Chamber (Catalog #27313)

\*These parts are guaranteed for 1 year against defects, leaks and breakage. Please do not return any warranty items without written authorization. As we cannot anticipate nor control conditions of product application, we do not warrant suitability or favorable results.

## Directions For Use

### TO OPEN THE CHAMBER:

1. Grasp chamber and ring clamp firmly with left hand opposite clamp handle.
2. Place right hand firmly over clamp handle and open slowly. The clamp can be easily removed (or replaced) from the chamber by extending the clamp to its full diameter.
3. Remove clamp.
4. Using both hands, remove the lid and trays. Always use both hands when handling lid, base, trays and the clamp as this will help prevent accidental spills.

### TO REASSEMBLE THE CHAMBER:

1. Check all O-ring surfaces to ensure they are free of any particles which could cause leaks. The O-ring should be cleaned periodically (see cleaning instructions). If the chamber leaks, inspect the O-ring and O-ring grooves to ensure they are clean and not damaged. If the chamber still fails to seal, check adjustment of the clamp; one or two turns of the adjustments are all that is necessary (see ring clamp instructions).
2. Position trays in base and ensure they are seated properly. Place lid on base.
3. Grasp clamp with left hand and center clamp at position where base and lid join together. Left hand should be held firmly against clamp and chamber. Slowly close clamp handle with right hand, ensuring clamp is centered. Chamber is now ready to be purged with gas.

### TO GAS THE CHAMBER:

1. Open both inlet and outlet ports (Tygon® tubing with white plastic clamps).
2. Attach inlet port Tygon® tubing to a source containing the desired gas mixture.

NOTE: Both port clamps must be open during this procedure. The chamber may rupture under excess pressure. Observe warning on chamber. WARNING: Do not exceed 2 psi above atmospheric pressure inside the Hypoxia Incubator Chamber.

3. We recommend purging the chamber at a rate of 20 liters/min (LPM) for 4 - 5 minutes.  
NOTE: Since flow rates and experimental conditions vary, we recommend that each investigator determines the time and frequency of flushing. A Single Flow Meter (Catalog #27311) is available from STEMCELL Technologies Inc.
4. After chamber has been purged, disconnect gas source.
5. Seal chamber by closing plastic clamps (white).
6. Put chamber at appropriate temperature either in a conventional continuous flow CO<sub>2</sub> incubator, an oven, or a warm room. If the chamber contains a large quantity of tissue culture plates, re-flush the system after 1 hour of incubation to remove any gases that may have been trapped in the plasticware.

## Notes and Tips

**HUMIDITY:** The chamber must be humidified to prevent excessive evaporation of culture medium. This can be accomplished by placing a Petri dish with 10 - 20 mL sterile water in the chamber (without a lid).

**RING CLAMP:** The ring clamp is the portion of the clamp that wedges the base and lid flanges together. As torque is applied to the adjustable bolt of the clamp handle, an inward radial force is created in the ring clamp. This force wedges the flanges together, causing them to squeeze the O-ring. The cam-action of the handles has been adjusted at the factory to provide a reliable air-tight seal. If the clamp needs to be adjusted, loosen the locking nut (found under handle) and turn the bolt clockwise to tighten or counterclockwise to loosen the clamp. After adjusting, tighten the locking nut.

**STACKING CHAMBERS:** Lightly lubricate the four indexing tabs of the lid using silicone grease. Center the base of the top chamber onto the lid of the bottom chamber tab, and while gently pressing down, rotate the upper chamber.

**CLEANING:** The chamber may be sterilized with 70% ethanol, 5% formalin, or sterilizing gas mixtures. When sterilizing with ethylene oxide the units should be placed in a vacuum chamber and de-gassed. The O-ring should be cleaned periodically to ensure a proper seal. When removing the O-ring be careful not to scratch the O-ring groove. Wipe the O-ring with a lint-free Kimwipe® and 70% ethanol. Then apply a minimum amount of silicone grease evenly and wipe off any excess. Grease is used only for proper seating of the O-ring and not for sealing.

**NOTE:** Avoid exposing the Hypoxia Incubator Chamber to solvents such as strong acids, organic solvents, and all bases. Polycarbonate is soluble in aromatic and chlorinated hydrocarbons. Avoid prolonged exposure to alcohol. Do not autoclave.

### HOW TO REPLACE THE HYPOXIA CHAMBER O-RING

1. Remove old O-ring, taking care not to scratch the O-ring groove.
2. Clean new O-ring with a lint-free Kimwipe® with 70% ethanol. Clean the O-ring groove with a Q-tip® or Kimwipe® and 70% ethanol.
3. Place new O-ring on top of the groove. Push down the O-ring with your thumbs approximately every 5 cm as you circle around the groove. Do not wipe the O-ring into the groove.
5. Put the lid on top of base, and push down hard. You can use your forearm to apply pressure on top of the lid.
6. Place the ring clamp around the chamber, and slowly close it, and leave it on for 30 seconds. The O-ring should now be properly placed in position. NOTE: The clamp may need to be adjusted.

### HOW TO CHECK FOR LEAKS

1. Seal the Hypoxia Incubator Chamber at room temperature (15 - 25°C), then place in a 37°C incubator for 1 hour.
2. Take the chamber out of the incubator. Place the Tygon® tubing close to your ear, then open up the plastic clamp and listen for gas being released. A "puff" indicates it is a good seal.

**STOPPING LEAKS:** If gas is leaking, the ring clamp may need to be readjusted. First loosen the nut and then turn the bolt on the clamp clockwise to tighten it. Use a 3/8 inch (7 - 9 mm) box wrench and flat head screw driver.

**NOTE:** Do not put silicone grease on the O-rings to make a seal.

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