

Gas Humidifier

The Bioptechs Gas Humidifier is a device placed into the flow path of ambient pressure gases (usually 5% CO2 in air) that are then passed to a cell culture micro-environment on a microscope. It works by heating water in a closed system vessel where a gas is introduced and bubbled in the heated water. The bubbled gas then rises to the surface of the water where it is further humidified by virtue of the 100% humidity that is maintained in the small airspace contained above the water. This is visible through the glass walls of the Humidifier. Condensation will form on the inner walls of the Humidifier then fall back into the heated water thereby maintaining a constant 100% RH within the airspace. The gas is then delivered to a vent tube and carried back down through the heated water so that condensation does not form in the tubing and RH is preserved. The gas then passes through a channel in the heated base thus preventing condensation in the short coupled outflow tube where it is immediately coupled to the cell chamber. During long term experiments where the water level may fall below the recommended level, additional sterile water can be added by simply removing the Lure plug on the top of the Humidifier then, using a Luer slip syringe, refill the unit to its recommended level. Gas tubing couplings are made of 14 gauge, 304 stainless steel needle-stock which is compatible with 1/16" or 1.5mm ID tubing without using special fittings.

Filling the bubbler:

- 1. Make sure that the water you intend to use is first pre-equilibrated with the gas concentration you intend to use before using the Humidifier.
- 2. The Humidifier should be start with 10ml of sterile water. If the level drops below the visible portion of the glass, additional water should be added, usually another 5ml.

Attaching and regulating the gas flow:

Using a gas regulator that is set to ambient pressure or a demand type regulator with a low differential delivery, attach tubing having a low gas diffusion rate to the outflow port on the regulator. Then pass the gas through a low flow peristaltic pump to regulate the gas flow. This method allows you to meter the flow at ambient pressure instead of having to deal with high pressure reduction into such a low-flow rate as required by cell chambers. Using short tubing attach the outflow from the pump to the humidifier inlet port. Then purge the lines and humidifier at a high flow rate for enough time to be assured that the humidifier is receiving the proper concentration of gas. Then attach the Humidifier to the cell chamber as close as possible and begin the necessary flow. It is recommended to have the electrical connection and gas supply line facing away from the cell chamber and the outflow tube directly coupled and facing the chamber.

Determining flow rate:

The best flow rate is the lowest flow rate that will maintain the pH or other gas requirement of your cells. Unless you have a special high-flow rate protocol you should experiment to find out what flow rate is best for your configuration. To experiment use a PH indicator such as Phenol Red in the media to achieve or keep the pH constant in your chamber prior to running any real world experiments. The best way to adjust your flow rate is to simply count the bubbles as they appear in the bubbler. Each bubble is approximately 16 microliter. As a rule bubble rates can vary from one bubble every second to one bubble every ten seconds. You will have to experiment to find what rate works best for your chamber and cell characteristics.

Cleaning:

Laboratory soap and water can be used for general cleaning and the entire unit can be disassembled and autoclaved for sterility if necessary. The glass tube simply pulls out of the two end caps. Assembly simply requires compressive pressure to re-insert the glass tube into the end caps. If the o-rings become damaged it can leak, Replacement o-rings are available from Bioptechs.



Humidifier with Delta T stage and Heated lid

Humidified gas out

Water fill port



Humidifier overview

Suggested water level

Gas in

Low water level

Power

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