

CO<sub>2</sub> incubators

QA

**Question: How can using the wrong type of water to provide in-chamber humidity cause corrosion in my CO<sub>2</sub> incubator?**

**Answer:**

You should only use sterile, distilled water in your CO<sub>2</sub> incubator that has a pH between 7 and 9.

Deionized (DI), reverse osmosis (RO), or ultrapure Type 1 water can be very aggressive, corroding incubator components.

Providing humidity for your cells is important, but the wrong kind of water can harm your incubator, corroding stainless steel, copper, glass, and other components. This is not a reflection on the quality of the materials used in the incubator, but rather the relative purity of the water. Increasingly we see laboratories with deionized (DI), reverse osmosis (RO), or ultrapure Type 1 water used in specialty instruments such as HPLC or spectroscopy or with cell culture media and additives.

But high purity water with low ionic strength is very aggressive to metals and glass over time. Water with a resistivity approaching or equal to 18.2 MΩ-cm contains very few ions. This highly pure water will actively pull ions from incubator components with higher ionic content, resulting in corrosion of those materials.



## Water provides humidity that is critical to maintain proper cell growth

Without high humidity (85 to 95%), evaporation of water from growth media leads to concentration of salts, minerals, and other nutrients. Because these ingredients are precisely balanced for your cell type, concentrations that are too high can be detrimental, even causing cell death. Evaporation is 4 times faster at 80% humidity than at 95%<sup>1</sup>. This is why fast humidity recovery is one of the most important parameters to consider when comparing CO<sub>2</sub> incubators. Humidity higher than 95% condenses back into water droplets that can nurture unwanted mold.

### Sterile distilled water is the best choice

Distilled water contains low amounts of ions, bacteria and other particles. After sterilization, it is appropriate for use in the CO<sub>2</sub> incubator. Many regions have tap water containing chlorine, a poor water choice due to chlorine's corrosive effects. Tap water may also have high mineral content that can lead to "scaling," or deposits, in the incubator chamber.

### Proper resistivity is critical to protect your incubator

Resistivity is the inverse of conductivity. Low ionic strength equals low conductivity/high resistance. DI and Type 1 water contain few ions and have very high resistivity. RO water can vary depending on the source quality, but also may have high resistivity. The common output quality of 18.2 MΩ-cm is too high for use in a CO<sub>2</sub> incubator because such water is aggressive against

stainless steel, copper, and glass. Water with low ion content/high resistivity will actively pull ions from these materials, resulting in corrosion which can lead to rust, flaking, or particulates. Thus, water used in the CO<sub>2</sub> incubator must contain some inorganic ions. A resistivity of 50 K-1 MΩ-cm (conductivity of 1-20 μS/cm) is recommended.

### pH should be neutral to basic

Highly pure water can be slightly acidic because carbon dioxide in the air readily dissolves in it, forming weak carbonic acid. This, too, can lead to corrosion. Check that your water has a pH of 7-9 to compensate for the carbon dioxide.

### Addition of weak sodium bicarbonate can help

Newer laboratories may only have access to DI or Type 1 water, or sterile distilled pre-bottled water. In this case, check that the resistance is 50 K-1 MΩ-cm, with pH 7-9. If your water falls outside these parameters, one option is to add a sterile solution of weak sodium bicarbonate to raise the pH and add ions<sup>2</sup>.

#### References:

- Esser P and Weitzmann L. Evaporation from cell culture plates. Thermo Fisher Scientific TILSPNUNCBU02 0111, 2011.
- Thermo Scientific Water Recommendation Reference Sheet. Thermo Fisher Scientific LT502X28, 2008.

## Characteristics of water types commonly used in CO<sub>2</sub> incubators

Purified water type	Characteristics	Resistivity (MΩ-cm)	Salts/ions	Use in CO <sub>2</sub> incubator
Tap water	May contain chlorine, bacteria, minerals, salts	Varies depending on location and system but generally <1	++	-
Reverse osmosis	Contains low levels of particles, bacteria, pyrogens, some inorganic ions and organics. Purity is directly dependent on feed water quality	Varies depending on input water quality, generally >4	+	+/-
Distilled (Type 2)	Contains low inorganic ions, particles, bacteria, and pyrogens	Generally 1-10	+	++
Deionized	Contains very low inorganic ions and dissolved gases. Does not reduce particulates or bacteria	Can be either RO (>4) or Type 1 (≤18.2)	-	-
Ultrapure (Type 1)	Contains no ions, very low organics (using UV) and very low pyrogens, nucleases and bacteria (using UV + ultrafilter).	≤18.2	-	-

### Summary

Corrosion in a CO<sub>2</sub> incubator can be caused by using water that is too pure. To ensure optimal growth conditions for your cells and long life for the CO<sub>2</sub> incubator, use only fresh sterile distilled water with low resistivity and pH of 7-9.

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