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## Best Practice to Re-Start a CO<sub>2</sub> Incubator

### Background

With the emergence of the SARS-CoV-2 virus and the associated COVID-19 pandemic, many research institutes have reduced work or even temporarily closed facilities.

Because CO<sub>2</sub> incubators are designed to grow cultured animal cells, they are also expert at growing associated normal flora and opportunistic microorganisms. As a result, it is possible that if not properly prepared for the shutdown, an inactive CO<sub>2</sub> incubator spent the quiet time growing such microorganisms. It is possible that you return to the lab after a weeks-long hiatus to find incubators full of mold or other fungi and bacteria.

Here we provide our best practice for re-starting a CO<sub>2</sub> incubator following weeks or even months of inactivity.

### Procedure for restart of CO<sub>2</sub> incubator if properly shut down

(Refer to “Best Practice for Temporary Shutdown of CO<sub>2</sub> Incubators”, March 2020)

**Note:** If not properly shut down, see next section.

1. Refer to the user manual for the specific model.
2. If the incubator has been idle for six months to one year, consider replacing the gas line air filters.
3. Clean the incubator exterior. Inspect the interior; it should be clean if the incubator has been unopened while idle, but if the incubator chamber has become dirty, clean as in step 5 of the shutdown procedure.
4. Spray the interior with 70% and allow to air dry.
5. If available, initiate the automated heat sterilization cycle, following the instructions in the user manual.
6. Following the sterilization, install a new HEPA filter if necessary.
7. Program the desired settings for temperature and CO<sub>2</sub> concentration. Fill the water reservoir with fresh sterilized distilled water.
8. If available, initiate the “Auto-Start” cycle.
9. After 24 hours of operation, confirm the CO<sub>2</sub> concentration using a handheld sensor such as a fyrite or handheld CO<sub>2</sub> sensor.
10. The incubator is now ready to receive cultured cells.

## **Procedure for restart of CO<sub>2</sub> incubator if not properly shut down**

(Follow these instructions if the incubator was not cleaned and disinfected prior to shutdown.)

1. Refer to the user manual for the specific model.
2. Inspect the interior. If there are any areas of visible microbial growth, remove this growth using wipes moistened in a quaternary ammonium disinfectant such as Conflit, Lysol No Rinse, or Fermacidal-D2.
3. Clean the incubator interior and exterior with mild dish soap and water. Clean the incubator shelves, shelf standards and parts, and the water reservoir. Rinse with clear sterilized distilled water and wipe dry using a clean, lint free towel.
4. Disinfect these parts using a quaternary ammonium disinfectant such as Conflit, Lysol No Rinse, or Fermacidal-D2. Alternatively, removable parts may be sterilized in an autoclave.
5. Spray the interior and parts with 70% ethanol and allow to air dry.
6. If the incubator has been idle for six months to one year, consider replacing the gas line air filters.
7. If available, initiate the automated heat sterilization cycle, following the instructions in the user manual, including removing the in-chamber HEPA filter if applicable.
8. Following the sterilization, install a new in-chamber HEPA filter if necessary.
9. Program the desired settings for temperature and CO<sub>2</sub> concentration. Fill the water reservoir with fresh sterilized distilled water.
10. If available, initiate the "Auto-Start" cycle.
11. After 24 hours of operation, confirm the CO<sub>2</sub> concentration using a handheld sensor such as a fyrite or handheld CO<sub>2</sub> sensor.
12. The incubator is now ready to receive cultured cells.

## **References**

1. Thermo Scientific Technical Memo: Best practice for shutting down a CO<sub>2</sub> incubator. March 17, 2020.
2. Thermo Scientific Technical Note. Proper care and maintenance for a cell culture incubator. Thermo Fisher Scientific TNCO2CARE 1217, 2017.
3. Thermo Scientific Smart Note. How can using the wrong type of water to provide in-chamber humidity cause corrosion in my CO<sub>2</sub> incubator? Thermo Fisher Scientific SNCO2WATER 0316, 2016.

## **For questions or for more information**

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