



## Operating the tissue-culture incubator's CO<sub>2</sub> regulator

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This protocol provides detailed instructions on the installation and operation of the tissue-culture incubator's CO<sub>2</sub> gas regulator. Two stage gas regulators allow for the depressurization of compressed cylinder gases to operational pressures. For the Sanyo MCO-15AC/17AC incubator a liquefied CO<sub>2</sub> gas cylinder (not a siphon or dip-tube cylinder) must be used together with a gas regulator rated at 3600 psiG (25 MPaG or 250 kgf/cm<sup>2</sup>G) on the primary side and 30 psiG (0.2 MPaG or 2.0 kgf/cm<sup>2</sup>G) on the secondary side. First stage decreases compressed cylinder pressure (1000 to 1900 psiG) by 90% while the second stage allows for the regulation of operational outflow pressure (3.5 to 4.3 psiG).

### Installation and operation

1. Ensure that the compressed CO<sub>2</sub> cylinder allows for gas withdrawal (normally grey coloured) and is not equipped with a siphon for liquid recovery (grey coloured with white vertical stripe).
2. Make sure compressed CO<sub>2</sub> gas cylinder is safely restrained to wall with either chains or brackets.
3. Remove compressed cylinder's valve protection hood and remove valve nozzle cap if present; point in safe direction and far from people to perform a half-second blowout by opening main cylinder valve. This should remove any dust or foreign debris from entering the gas regulator once installed.
4. Connect the corresponding gas regulator port (regulator supply) to compressed cylinder nozzle using Teflon tape to ensure proper fit and prevent leaks.
5. Connect the corresponding gas regulator port (regulator outflow) to downstream tubing leading to the tissue-culture incubator.
6. Close off all valves downstream of CO<sub>2</sub> regulator.
7. Turn main regulator valve knob (large knob) counterclockwise until it is felt to rotate freely (takes a couple of turns only). CO<sub>2</sub> gas regulator is now completely shut off.
8. Slowly open main compressed cylinder valve until first stage dial (the one on the right) indicates maximum cylinder pressure (usually between 1000 and 1900 psiG).





9. Slowly turn main regulator knob (large knob) clockwise until outflow gas pressure between 3.5 to 4.3 psiG is reached (0.03 MPaG or 0.3 kgf/cm<sup>2</sup>G) as indicated by second stage dial (the one on the left). Regulator is now primed, but no CO<sub>2</sub> has flowed into incubator supply line yet.

**HAZARD WARNING:** Regulator outflow pressures in excess of 3.5 to 4.3 psiG may cause disconnection of internal piping inside the CO<sub>2</sub> incubator, which will result in leakage of CO<sub>2</sub> gas into the laboratory atmosphere. Elevated levels of CO<sub>2</sub> may lead to asphyxiation and death.

10. Using a mild neutral soap solution inspect all connections (but not regulator dials and main housing) for gas leaks (bubbles). Take corrective measures if found, otherwise rinse with distilled water and wipe dry with 70% ethanol-soaked gauze.
11. To supply CO<sub>2</sub> to tissue-culture incubator open downstream valve (small black valve located on outflow port and to the left of the regulator), a hiss should indicate CO<sub>2</sub> supply lines linking regulator to incubator have been filled.

### Shutdown procedure

1. Close all valves downstream of regulator.
2. Close main valve of compressed cylinder.
3. Turn regulator main knob (large knob on center) counterclockwise a couple of turns until it rotates freely.
4. Regulator is now completely closed. If necessary, disconnect from compressed cylinder.

### References

1. SANYO MCO-17AC/15AC CO<sub>2</sub> Incubator Instruction Manual. Oct 1<sup>st</sup>, 2005. 7FB6P101262006. SANYO Electric Co., Ltd. 5-5, Keihan-Hondori 2-Chrome Moriguchi City, Osaka 570-8677 Japan.





## Revision history

- 1.0 Original document.
- 2.0 Added references and formatting changes.
- 3.0 Units changed from MPaG to psiG and reference to Sanyo manual added.

