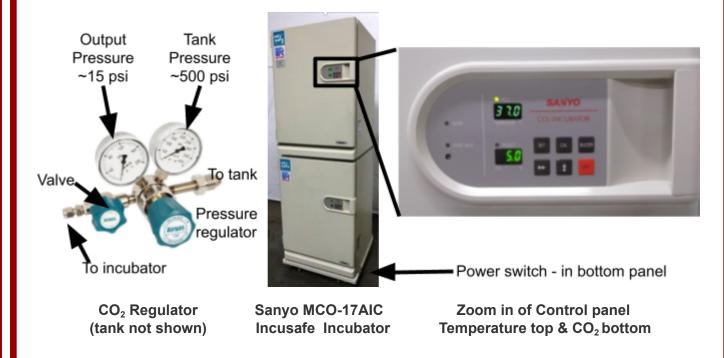
## CO<sub>2</sub> Incubators



Cells are typically grown at  $37^{\circ}$ C in an atmosphere of 5-10% CO<sub>2</sub> because the medium used is buffered with sodium bicarbonate/carbonic acid and the pH must be strictly maintained (7.2-7.5). Incubators are also used to block visible light, which can have an adverse effect on cells and components of the cell culture media.

- Cells should be left out of the incubator for as <u>little</u> time as possible
- Incubator doors should not be opened for very long
- Sterile technique must strictly be used before entering the incubator (70% Ethanol gloves and wipe surfaces of anything that goes in to avoid growing contaminants in the incubator.
- Label everything!!! Your name, cell type, cell passage number, passage ratio, and date

Step 1: Make sure culture flasks have loosened (or filtered) caps to allow for sufficient gas exchange.

Step 2: Check to make sure that the water pan is full. The water pan is located inside the incubator at the bottom. If low or empty, fill with autoclaved or filter **sterilized RO water (not diWater**, diWater can corrode metals). This ensures that the humidity is maintained to prevent evaporation. Humidity should be 90%+. RO water is located at the sink on the side spigot.

Step 3: Check that the  $CO_2$  is at 5% and the temperature is at 37 °C on the front of the door. If it is off, it can take ~24-48 to stabilize the temperature, once stable at 37 °C, then set and turn on the  $CO_2$ .

Step 4: Check the regulator to be sure the tank is not nearing empty. It will fall from 500 psi to around 200-300 psi and stay there for a few days before going completely empty.