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## Operating the gassing manifold

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**We use this protocol and it's working**

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## Abstract

### Standard operating procedure for using the gassing manifold

The gassing manifold is used for replacing the headspace of vials with N<sub>2</sub> (or N<sub>2</sub>:CO<sub>2</sub>) or for degassing solutions. It is useful for preparing anoxic incubations, media and buffers before bringing them in closed vials into the anoxic chamber. The manifold is equipped with an electronic controller that automatically switches between the gas inlet and the vacuum pump and performs multiple cycles of headspace flushing.

## Materials

### MATERIALS

✕ Disposable needles (large enough to penetrate through the vial stoppers)

✕ Syringe filters (0.1-0.2 µm)

## Troubleshooting

## Safety warnings

- ! This system works with pressurised gasses. Make sure you understand and know how to work with high pressure gasses.
- Always wear safety goggles when operating.
- Always make sure the vials are capped tightly in addition to the stoppers, either with a screw-cap lid or a crimped aluminium cap.



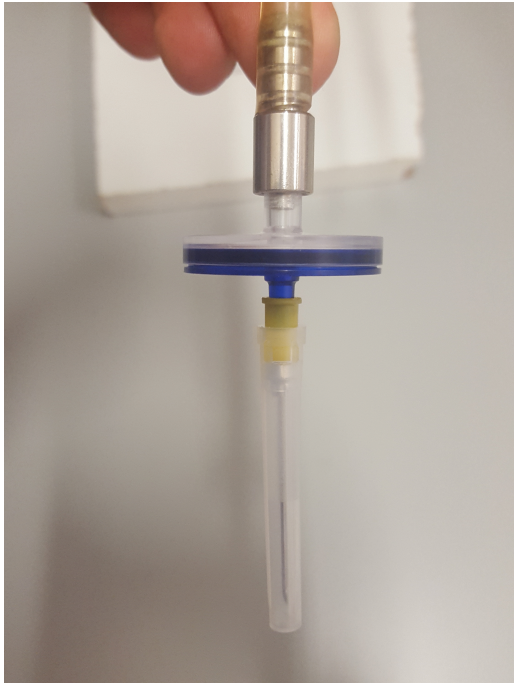
## Before start

- Make sure all tubes have syringe filters (0.1-0.2  $\mu\text{m}$ ) attached to their Luer fitting.
- Make sure the pump has sufficient oil in it (watch the window in front of the pump where the oil level is visible).
- Make sure the gas switching valve is pointing to the correct gas you wish to use (in case more than one is connected).
- Only vials capped with a flexible rubber stopper can be used with this device.

- 1 Attach new needles to the syringe filters. If working with sensitive samples, the syringe filters should also be changed to avoid contamination.

#### Note

The selected needles must be thick enough to penetrate the stoppers, but thin enough as to not break the stoppers.



- 2 Attach the vials by piercing the stoppers with the attached needles.

#### Safety information

The needle should never touch the liquid inside the bottle!





- 3 Turn on the main pressure knob almost all the way through.

#### Safety information

Never force the knob open or close beyond its stopping point!



- 4 Turn on the pressure adjusting knob. Usually this is set to be between 0.5 and 1 bar.

#### Safety information

- Never force the knob open or close beyond its stopping point!
- Whatever the pressure gauge shows will also be the level of overpressure in the vial!

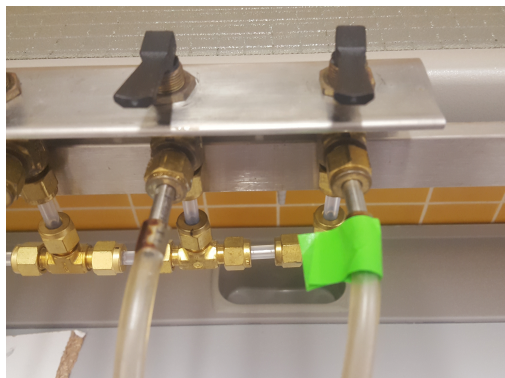
- 5 Turn on the pump.



- 6 Open the valves of the tubes to which vials were connected.

#### Note

Keep all other valves closed.



- 7 Flip the switch to turn on the controller.



- 8 Wait and allow the vials to be flushed properly.

#### Note

The number of flushing cycles needed to turn the vials anoxic will depend on their content. Empty vials only need a about 3-5 cycles to become anoxic but vials with liquids will require longer to degas completely. This should be tested when using a new set-up of vials.

- 9 Turn off the controller switch and allow the vials to reach overpressure.

### Note

What the pressure gauge above the controller shows is the overpressure in the vials



- 10 Turn off the pump.
- 11 Close the valves and remove the vials.
- 12 Turn off the main pressure knob.

### Safety information

Do not use excess force!

- 13 Turn off the main pressure adjusting knob.



### Safety information

Do not use excess force!

- 14 Release the remaining pressure in the system by quickly opening and closing one of the tube valves.