

Setting up the Pneumatic Primer

Principle:

The Pneumatic Primer is a pressure regulator that flows inert gas at a low pressure through the SynVivo devices. The Pneumatic Primer consists of an inlet connector that connects the microfluidics device to the pressure regulator (**Figure 1A**). On the opposite side of the pressure regulator box, a metal male connector (**Figure 1B**) will join the pressure regulator to nylon tubing containing inert gas (**Figure 1C**; use $\frac{1}{4}$ inch or 0.635 cm outer diameter; $\frac{11}{64}$ inch or 0.436 cm inner diameter nylon tubing).



Figure 1. A top view of the Pneumatic Primer, containing an inlet connector for the device (A), a metal male connector to the gas line (B), and nylon tubing that connects to the gas tank (C).

If the source of the inert gas is industrial grade, the gas may contain particles that could potentially contaminate the device during perfusion. The $\frac{1}{4}$ -inch outer diameter, Push-in Serviceable Filter that comes with the Pneumatic Primer kit can be used to filter any particles from the flow of gas (**Figure 2A**). Three replaceable filters are also included with the Pneumatic Primer kit, and the 5-micron filters should be replaced every 3-6 months depending on usage.

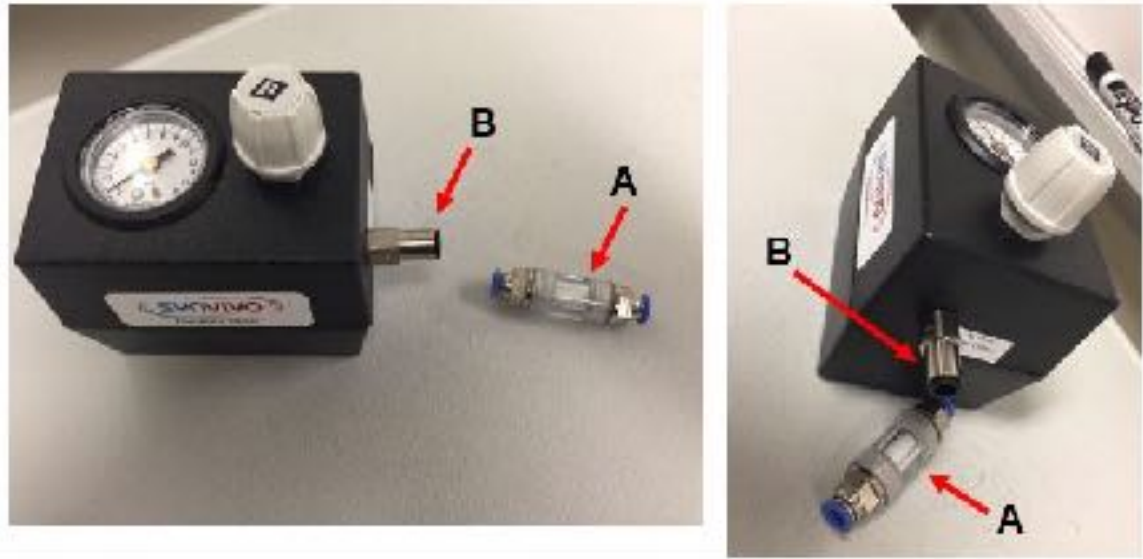


Figure 2. Visual comparison of the ¼ inch (0.635 cm) outer diameter (OD) push-in serviceable filter (A), to the metal male connector on the Pneumatic Primer Box (B).

Equipment:

- Pneumatic Primer (SynVivo 205001)
 - Inlet connector
 - LEGRIS ¼ inch (0.635 cm) metal male connector (Grainger 1PFG1)
 - ¼ inch (0.635 cm) outer diameter Push-in Serviceable Filter (Industrial Specialties Mfg. PIF25-14-PP-5)
 - 3 porous polypropylene elements (5 micron; Industrial Specialties Mfg. PIF25-RF-PP-5)
- Multiple port manifold for the Pneumatic Primer (SynVivo 207001; optional)
- Nylon tubing (1/4 inch or 0.635 cm OD; 11/64 inch or 0.436 cm ID; Grainger 2VDL8)
- Gas cylinder, pressure regulator, and adapters for ¼ inch (0.635 cm) OD nylon tubing
- SynVivo microfluidics device
- Tygon tubing (0.02 inch or 0.0508 ID; 0.06 inch or 0.1524 cm OD; SynVivo 201005)
- Slide clamps (SynVivo 202001)



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- 24-gauge blunt-tipped needles (0.5 inch or 1.27 cm long; SynVivo 204003)

Protocol:

1. The user is responsible for purchasing the gas cylinder, the appropriate pressure regulator, tubing adapters to fit the specifications of the nylon tubing (1/4 inch or 0.635 cm OD; 11/64 inch or 0.436 cm ID), and the nylon tubing itself (**Figure 3A**).
2. The gas pressure flowing from the tank and into the Pneumatic Primer will be set at 20 psi or 137.9 kPa (**Figure 3B**), but **do not turn on the gas until all connections are securely fastened**.
3. The serviceable filter can be installed in between the pressure regulator on the gas cylinder and the Pneumatic Primer (**Figure 3C**), using a piece of nylon tubing.
 - a. Connect one end of the nylon tubing to the pressure regulator, using adapters that are appropriate for the inner and outer diameters of the tubing.
 - b. Connect the other end of the nylon tubing to the filter.
 - i. Push in one of the blue connectors on the filter, and insert the nylon tubing into the blue connector.
 - ii. Release the blue connector, which will tighten around the nylon tubing and form a secure connection.
4. A second piece of nylon tubing joins the air filter to the Pneumatic Primer by the metal male connector attached to the Pneumatic Primer Box (**Figure 3D**).

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- a. Connect the nylon tubing to the air filter, as described in Step 3b.
5. Connect the nylon tubing to the Pneumatic Primer.
 - a. By pushing in on the plastic end of the male connector, the nylon tubing can then be inserted into the connector.
 - b. Releasing the plastic end will tighten the connection and secure the nylon tubing to the Pneumatic Primer box.
 6. With the knob of the Pneumatic Primer set at “0”, the gas can now be turned on and set at 20 psi or 137.9 kPa. Listen carefully for any gas leaks.

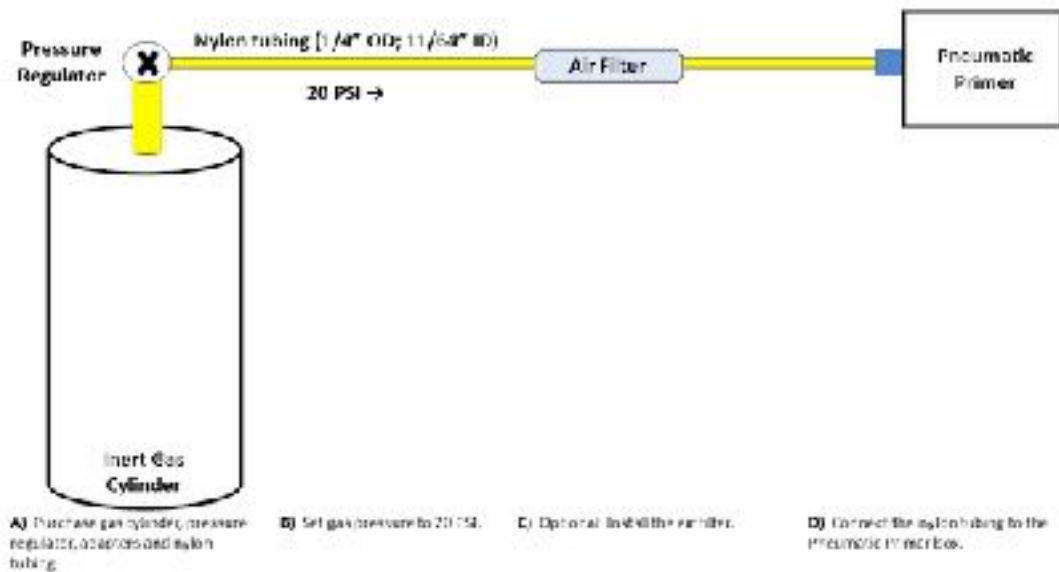


Figure 3. Schematic for connecting the Pneumatic Primer to a source of inert gas.

7. Once the connections have been made between the gas cylinder and the Pneumatic Primer box, the microfluidics device can be connected to the white inlet connector (**Figure 4**).

8. Unclamp one of the pieces of Tygon tubing that is connected to the microfluidics device.
9. Slide a 24-gauge, blunt-tipped needle into the Tygon tubing, so that the needle is inside the tubing.
10. Screw the red casing of the needle into the white inlet connector of the Pneumatic Primer box. Make sure the connection is secure.

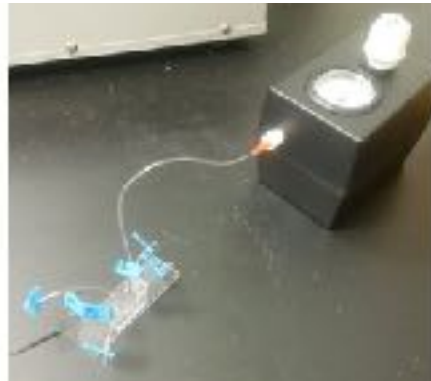


Figure 4. Schematic for connecting the microfluidics device to the Pneumatic Primer.

11. Turn the white knob on the Pneumatic Primer clockwise, until gas is flowing and the pressure on the gauge reads between 5-7 psi (34.47–48.26 kPa).
12. Listen carefully to make sure no gas is leaking out of any connections.
13. Proceed with priming as described in the SynVivo protocol.

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14. Turn the white knob on the Pneumatic Primer counterclockwise to turn off the flow of gas.

15. If using the Multiple Port Manifold, attach the manifold to the Pneumatic Primer (**Figure 5A**).

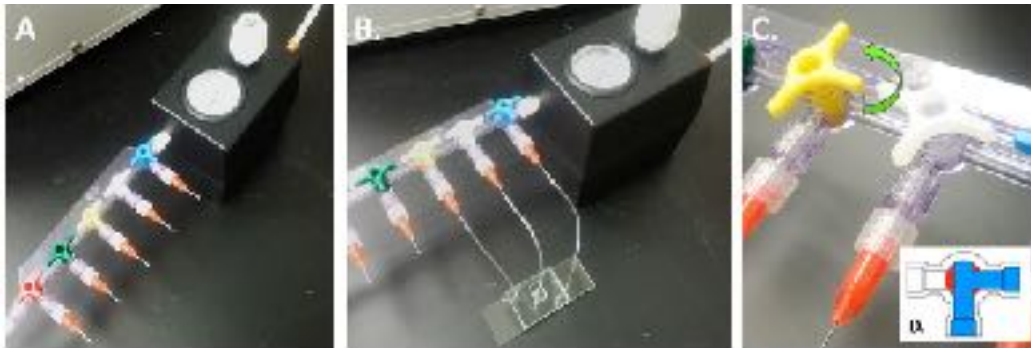


Figure 5. The Multiple Port Manifold is connected to a Pneumatic Primer (A). All 3 ports of a microvascular network device with a 3D tissue chamber are connected to the manifold (B). (C) Turn the 3-way valve counterclockwise on the T port, so that the flow of gas is directed into the last port of the device (D; inset graphic from <https://valveman.com/blog/understanding-tport-vs-lport-directional-flows/>).

16. Connect the devices to the manifold using the red needles (**Figure 5B**).

17. Turn the last 3-way valve that is connected to the device in a counterclockwise manner, so that the flow of gas is directed into the last port of the device (**Figure 5C**).

18. Turn the white knob on the Pneumatic Primer clockwise, until gas is flowing and the pressure on the gauge reads between 5-7 psi (34.47–48.26 kPa).

19. Listen carefully to make sure no gas is leaking out of any connections.



Appendix

20. Proceed with priming as described in the SynVivo protocol.

21. Turn the white knob on the Pneumatic Primer counterclockwise to turn off the flow of gas.