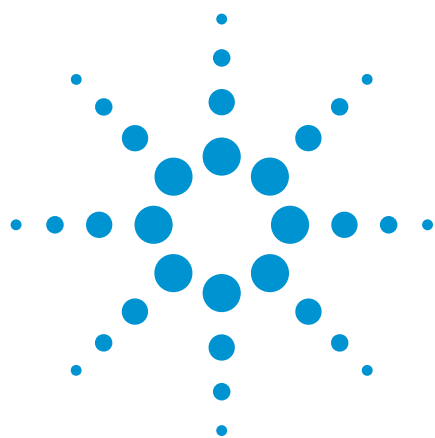




A Technical Guide to Agilent Positive Pressure Manifold 48 and 96 Processors

Technical Overview

General description

Positive pressure processing of cartridges and 96-well plates, such as solid phase extraction (SPE), supported liquid extraction (SLE), and filtration (protein precipitation), offers many advantages over traditional vacuum processing. Conventional vacuum manifolds pull liquid from the bottom of a cartridge or 96-well plate. When faster flowing cartridges run dry, the vacuum will follow the path of least resistance through the empty cartridges, thereby slowing flow through the remaining cartridges. This can lead to variation in processing times and irreproducible results. For cartridge-style vacuum manifolds, caps or plugs are necessary to block unused ports. Stopcock valves are often implemented, adding significant time to sample processing due to valve opening and closing. Vacuum manifolds also lack the processing power for tough-flowing, viscous samples since the maximum pressure is limited to less than atmospheric pressure (approximately 15 psi).

Agilent positive pressure manifold (PPM) 48 and 96 processors are excellent alternatives for sample processing (Figures 1 and 2). The PPM-48 and PPM-96 have unique restricted-flow ports to create consistent gas flow through every channel, even when channels are not being used, or run dry. This ensures reproducibility from row-to-row and cartridge-to-cartridge regardless of cartridge or well contents. The PPM-48 can process up to 48 sample preparation cartridges simultaneously, allowing for higher throughput than traditional 24-cartridge vacuum manifolds. Cartridge racks are available in 1, 3, and 6 mL sizes, which support multiple sample preparation products and laboratory protocols. Due to its unique design, the need for stopcock valves and caps or plugs is also eliminated. The PPM-96 is compatible with 96-well plates, Agilent VersaPlate formats, and 1 mL tabless cartridges. A 1 mL tabless cartridge holder (p/n 5191-4119) is required when using tabless cartridges. In addition, the PPM-48 and PPM-96 can efficiently process more viscous samples since the supplied gas pressure (50-100 psi) is much higher than traditional vacuum manifolds.



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The PPM-48 and PPM-96 are easily incorporated into any laboratory. The modular rack feature enables simple stacking of cartridge and collection racks (PPM-48) or 96-well plates and collection plates (PPM-96). Cartridge and collection racks are physically keyed to fit onto the sliding platform, and onto each other in one orientation only. This ensures stability during platform movement, and reduces the potential for rack orientation mix-ups. The processors are self-adjusting to accommodate different size 96-well plates, cartridges, test tubes, and even autosampler vials without the need for extra spacers. This makes Agilent PPM processors an easy and efficient solution for sample processing.



Figure 1. Agilent positive pressure manifold 48 processor.



Figure 2. Agilent positive pressure manifold 96 processor.

Protocol-specific instructions

Note: Please read the user manual before installation for more detailed information, safety hazards, and user warnings.

The following sections provide example protocols for solid phase extraction and filtration (protein precipitation) workflows for the PPM-48 and PPM-96. Refer to Figures 3 and 4 for labeled PPM components.

Note: Each of these protocols should serve as a general guide for how to operate the PPM processor. Flow rates, sample/solvent types and volumes, number of extraction steps, and dry times may differ depending on analyte, matrix, and laboratory protocol. Pressure settings and adjustments for the processor will vary depending on the number of rows in use (PPM-48), sample/solvent type, cartridge/well size, sorbent type and bed mass, specific sample preparation products used, and so forth.

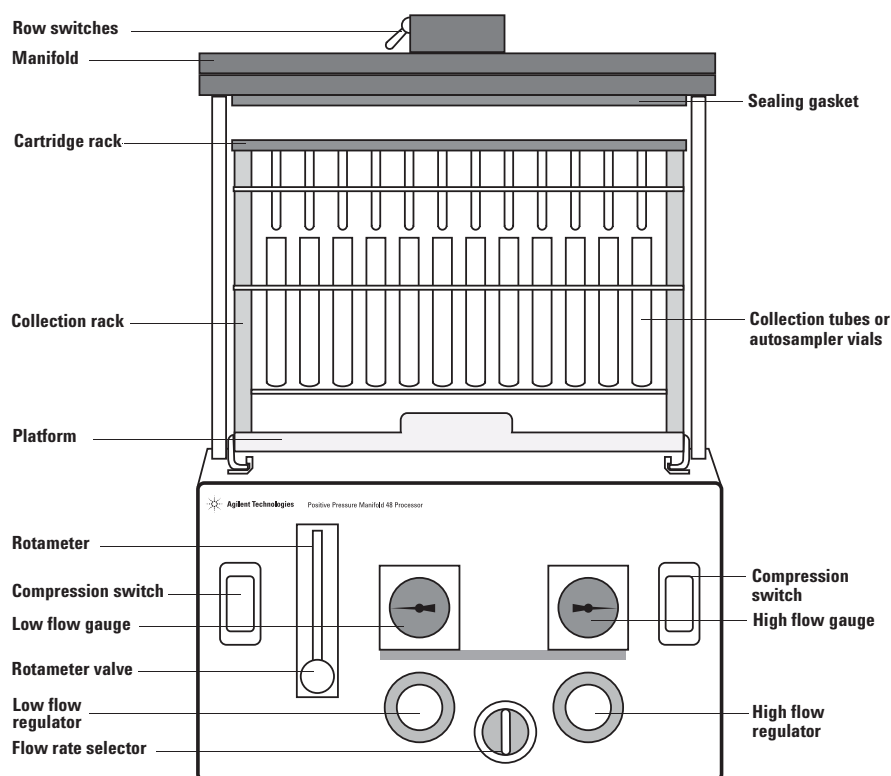


Figure 3. Agilent positive pressure manifold 48 processor and associated components.

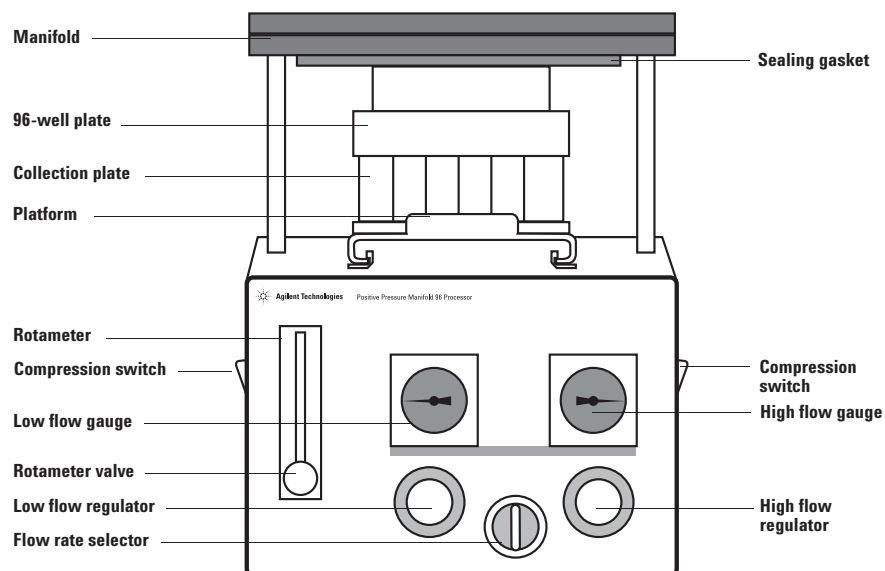


Figure 4. Agilent positive pressure manifold 96 processor and associated components.

Reagents/chemicals used

HPLC-grade methanol and acetonitrile were bought from Honeywell (Muskegon, MI, USA). Water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany). Formic acid was procured from Sigma-Aldrich Corporation (St. Louis, MO, USA). Plasma was from Biological Specialty Corporation (Colmar, PA, USA)

Materials used

- Agilent PPM-48 processor (p/n 5191-4101)
- Agilent PPM-96 processor (p/n 5191-4116)
- 1 mL SPE cartridge rack for PPM-48 (p/n 5191-4102)
- Waste rack with three waste bins for PPM-48 (p/n 5191-4112)
- Collection rack, 12 × 32 mm autosampler vials, PPM-48 (p/n 5191-4109)
- Holder for plates, PPM-96 (p/n 5191-4120)
- Single well waste plate for PPM-96 (p/n 5191-4121)
- Agilent Bond Elut Plexa, 30 mg, 1 mL cartridge (p/n 12109301)
- Agilent Bond Elut Plexa 96 round-well plate, 1 mL, 30 mg (p/n A4969030)
- Agilent Captiva EMR—Lipid, 1 mL cartridge (p/n 5190-1002)
- Agilent Captiva EMR—Lipid, 96-well plate (p/n 5190-1000)
- Agilent Captiva 96-deep well collection plate, 1 mL (p/n A696001000)
- Agilent Captiva pierceable 96-well collection plate cover (p/n A8961007)
- Agilent amber screw-top glass vials with write-on spot, certified, p/n 5182-0716
- Agilent bonded screw cap, PTFE/red silicone septa, p/n 5190-7024
- Eppendorf pipettes and repeater

Example Protocol for Bond Elut Solid Phase Extraction (SPE)

Note: This is a general guideline. Your protocol may vary based on analyte, matrix, and SPE sorbent.

- **PPM-48:** Agilent Bond Elut Plexa Polymeric SPE 30 mg, 1 mL cartridges (p/n 12109301)
- **PPM-96:** Agilent Bond Elut Plexa Polymeric SPE 30 mg, 1 mL 96 round-well plate (p/n A4969030)

PPM-48 and PPM-96

1. **Sample pretreatment:** Add 400 µL water with 0.5% formic acid to 100 µL plasma, vortex, and centrifuge.
2. **PPM-48:** Load 1 mL SPE cartridges into a 1 mL cartridge rack. Place a waste rack and waste bin under the cartridge rack.
PPM-96: Load a 1 mL SPE 96-well plate onto a single well waste tray fitted with a holder for plates.
3. Place the cartridge/plate stack onto the platform.
4. Set the low flow pressure to 3-4 psi.
 - a. **PPM-48 only:** Use the row switches to open the desired row valves.
 - b. Turn the flow rate selector from **Off** to **Low Flow**.
Note: It is easier to adjust the pressure with the gas flowing through the system (**Low Flow** or **High Flow**). However, raising or lowering the manifold should occur when there is no flow (**Off**).
 - c. Pull out the low flow regulator knob, and turn clockwise until the desired pressure is achieved.
 - d. Push the regulator knob in to lock.
 - e. Without closing the rotameter, adjust the rotameter valve to achieve the desired flow rate. It is recommended to start with a low flow rate, then increase as necessary.
Caution: To avoid damage to the processor, never shut off the rotameter. Only use the flow rate selector to turn the gas flow on or off.
 - f. Turn the flow selector to **Off**.
5. **Condition:** Add 1 mL methanol (MeOH) with 0.5% formic acid to each cartridge/well.
Caution: If spillage occurs during any step, wipe immediately before proceeding to the next step.
6. Slide the platform back until the cartridge plate stack is positioned under the manifold and reaches a stop.
7. Ensure that the flow rate selector is set to **Off**, and lower the manifold by simultaneously pressing down on the two compression switches.
8. Turn the flow rate selector to **Low Flow**.
9. Adjust the rotameter valve to achieve the desired liquid flow rate (for example, 1-2 mL/min).

10. After all the liquid has eluted from the cartridges/wells, turn the flow rate selector to **Off**.
 11. Raise the manifold by simultaneously pressing up on the two compression switches.
 12. Slide the platform forward until it stops.
 13. Repeat steps 5-12 for the following workflow steps:
 - **Equilibrate:** 1 mL water with 0.5% formic acid
 - **Load*:** Add pretreated sample
 - **Wash:** 1 mL 30% MeOH in water
- * Depending on the specific protocol, samples may be loaded using gravity or at the recommended flow rate (protocol dependent) with positive pressure (**Low Flow**, 3-4 psi).
14. **Dry:** After elution of the wash solvent (step 10), turn the flow rate selector from **Low Flow** to **High Flow**, and adjust to the desired pressure (50-80 psi). Dry for approximately 5 minutes. Turn the flow rate selector to **Off**. Raise the manifold, and slide the platform forward.
 15. **Collection:** Replace the waste plate/rack with the appropriate collection plate/rack with test tubes or autosampler vials. Add elution solvent (250 µL MeOH) to each cartridge/well. Elute using gravity or positive pressure (**Low Flow**) according to the protocol. Repeat with another 250 µL MeOH, combining both aliquots.
 16. Sample extracts may be diluted, dried down and reconstituted, or directly analyzed based on specific protocol requirements.

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Example Protocol for Protein Precipitation

Note: This is a general guideline. Your protocol may vary based on analyte, matrix, and sorbent.

- Agilent Captiva EMR—Lipid, 1 mL cartridges (p/n 5190–1002)
- Agilent Captiva EMR—Lipid, 96-well plate (p/n 5190–1000)

PPM-48 and PPM-96

1. **PPM-48:** Load 1 mL Captiva EMR—Lipid cartridges into a 1 mL cartridge rack. Place a collection rack with test tubes or autosampler vials under the cartridge rack.
PPM-96: Load a 1 mL Captiva EMR—Lipid 96-well plate onto a 96-well collection plate.
2. Place the cartridge/plate stack onto the platform.

3. Set the low flow pressure to 3-4 psi.
 - a. **PPM-48:** Use the row switches to open the desired row valves.
 - b. Turn the flow rate selector from **Off** to **Low Flow**.
Note: It is easier to adjust the pressure with the gas flowing through the system (**Low Flow** or **High Flow**). However, raising or lowering the manifold should occur when there is no flow (**Off**).
 - c. Pull out the low flow regulator knob and turn clockwise until the desired pressure is achieved.
 - d. Push the regulator knob in to lock.
 - e. Without closing the rotameter, adjust the rotameter valve to achieve the desired flow rate. It is recommended to start with a low flow rate, then increase as necessary.
Caution: To avoid damage to the processor, never shut off the rotameter. Only use the flow rate selector to turn the gas flow on or off.
 - f. Turn the flow rate selector to **Off**.
4. Add 400 μ L ACN with 1% formic acid to each cartridge/well.
Caution: If spillage occurs during any step, wipe immediately before proceeding to the next step.
5. Add 100 μ L of plasma to each cartridge/well.
6. Aspirate and dispense with pipette tips five times.
7. Wait 1 minute for complete protein precipitation.
8. Slide the platform back until the cartridge rack/plate stack is positioned under the manifold, and comes to a stop.
9. Ensure that the flow rate selector is set to **Off**, and lower the manifold by simultaneously pressing down on the two compression switches.
10. Turn the flow rate selector to **Low Flow**.
11. Adjust the rotameter valve to achieve the desired liquid flow rate (1 drop/3-5 seconds for Captiva EMR—Lipid).
12. Maintain the appropriate flow throughout elution, opening the rotameter valve more as faster flowing cartridges/wells finish eluting.
13. After elution of all cartridges/wells, turn the flow rate selector to **High Flow** and adjust to the desired pressure (50-80 psi). Dry for approximately 1 minute.
14. Sample extracts may be diluted, dried down and reconstituted, or directly analyzed based on specific protocol requirements.

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Conclusions

Agilent PPM-48 and PPM-96 processors are simple and efficient alternatives for processing Bond Elut SPE, Chem Elut SLE, Captiva filtration, and related sample preparation products. The self-adjusting manifolds of the PPM-48 and PPM-96 can accommodate multiple cartridge sizes (1, 3, and 6 mL) and 96-well plates (1 and 2 mL) without the need for special spacers. The flow-restricted ports provide uniform flow rates leading to less processing time variation and more reproducible results. Using a forced gas supply, such as nitrogen, the processors can apply pressures much higher than a traditional vacuum manifold, making the PPM-48 and PPM-96 excellent choices for highly viscous samples. The PPM-48 can accommodate up to 48 samples at once, allowing for higher throughput compared to traditional vacuum manifolds, saving both time and money. An optional autosampler vial collection rack (p/n 5191-4109) eliminates the need for the final transfer step from test tube to vial, and provides another cost-savings opportunity. Due to their versatility, simplicity, and ease-of-use, Agilent PPM-48 and PPM-96 processors can easily be incorporated into most existing workflows, and make excellent additions to any sample preparation laboratory.

For More Information

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