



Priming as New or Dry system using undegassed solvents.

1. Fill the solvent bottles with Mobile Phase
2. From the **Status Page** turn the degasser **On**.*
3. Perform a dry prime until solvent flows from each of the four lines.
4. Starting with the organic solvent, perform a 3 minute **Wet Prime** on each line separately at a flow rate of 5.0 mL/min.**
5. Once all lines are primed, set the flow rate to 0.0 mL/min with the degasser on for 10 minutes.
6. Start the flow with the initial conditions of your chromatographic method. Equilibrate with 10 column volumes of solvent.
7. Check the **Pressure Ripple** on page two of the **Status Page**. The ripple should be less than 2% of the system pressure. (For systems pressures above 1000 psi, repeat **Wet Prime** if the ripple is too high)
8. After the column is equilibrated, **Purge** the autosampler. (Initial conditions)
9. Run samples.

* The degasser should never be left on with no flow for long periods of time (I.e. overnight)

** Caution: To avoid precipitating salts in the Separations Module, use an intermediate solvent such as water when you change from buffers to high-organic content solvents.

Priming an idle system using existing mobile phase

1. From the Status Page turn the degasser **on**. *
2. For each line that will be used, starting with the organic solvent, perform a 1.5 minute **Wet Prime** at a flow rate of 5.0 mL/min. **
3. Once all lines are primed, set flow rate to 0mL/min with degasser on **continuous** for 10 minutes.
4. Start the flow with the initial conditions of your chromatographic method. Equilibrate with 10 column volumes of solvent.
5. Check the **Pressure Ripple** on page two of the **Status Page**. The ripple should be less than 2% of the system pressure. (for system pressures above 1000psi, repeat **Wet Prime** if the ripple is too high)
6. After the column is equilibrated, **Purge** the autosampler. (initial conditions)
7. Run Samples.

* The degasser should never be left on with no flow for long periods of time. (i.e. overnight)

** Caution: To avoid precipitating salts in the Separations Module use an intermediate solvent such as water when you change from buffers to high-organic content solvents.

Changing the Mobile Phase

1. Fill all of the solvent bottles with mobile phase.
2. For each line that will be used, starting with the organic solvent, perform a 3 minute **Wet Prime** at a flow rate of 5.0 mL/min. **
3. Once all of the lines are primed, set the flow rate to 0 mL/min with the degasser **on** for 10 minutes. *
4. Start the flow with the initial conditions of your chromatographic method. Equilibrate with 10 column volumes of solvent.
5. Check the **Pressure Ripple** on page two of the **Status Page**. The ripple should be less than 2% of the system pressure. (for system pressures above 1000psi, repeat wet prime if the pressure is too high)
6. After the column is equilibrated, **Purge** the autosampler. (initial conditions)
7. Run Samples.

* The degasser should never be left on with no flow for long periods of time. (i.e. overnight)

** Caution: To avoid precipitating salts in the Separations Module use an intermediate solvent such as water when you change from buffers to high-organic content solvents.

Note: When first starting to equilibrate your column, one or two Bubble Detects is normal. The system is detecting any small bubbles and using its' automatic algorithm to clear the bubbles.

Note: The volume of each solvent line in a 2695 including the vacuum degasser chamber is 5.5 mL. This volume must be considered when priming the system and changing to a different mobile phase.

Note: The degasser can be turned off, the flow rate stopped and the lamp turned off (if controlling the detector through the Alliance) in a Shutdown Method.

Priming the Seal Wash Pump (when needed)

1. Press the **Diag** screen key in the Main Menu screen. The Diagnostic screen appears.
2. Attach the syringe adapter (from the Start-up Kit) to the syringe.
3. Remove the solvent filter from the plunger seal wash inlet or remove the plunger seal wash waste line from the waste container. (Either pushing solvent through it or drawing solvent through it can be used to prime the plunger seal wash pump)
4. Fill the syringe with plunger seal wash solvent* and attach the syringe adapter to the end of the plunger seal wash line.
5. Press the **Prime Sealwash** Screen key and then press the **Start** screen key. The plunger seal wash procedure begins. Push on the syringe to push seal wash solution through the system or draw on the syringe to pull solvent through the plunger seal wash pump.
6. When solvent flows out of the plunger seal wash line or into the syringe (the pump will run quieter when it is primed) press the **Halt** screen key.
7. Reinstall the solvent filter and place the seal wash inlet line back into the plunger seal wash reservoir or place the plunger seal wash waste line back into the waste container. To finish the priming, press the **Start** screen key again.
8. Press **Close** to return to the diagnostics screen.

* Choose a Seal Wash solution that has high solubility with salts and /or buffers used in the mobile phase. (Usually a solution of 80 - 90% aqueous, 20 - 10% methanol)

Priming the Needle Wash Pump (daily or weekly)

1. Make sure Needle Wash line (Green Line) is in its appropriate bottle.
2. Press the **Diag** screen key in the Main screen. The Diagnostics screen appears.
3. Press the **Prime NdlWsh** screen key. The 30-second needle wash procedure begins. If solvent does not flow out of the waste line (the yellow line) press the **Start Again** screen key.

Note: Choose a Needle Wash solution that your sample is soluble in. Buffered solutions or a mobile phase modifier such as TFA are not recommended. If these solutions are necessary they must be flushed from the instrument.