

CombiFlash[®] Rf 75

Quick Start Guide

Instruction Sheet 60-5233-506
March 30, 2009

Overview

These instructions supplement the *CombiFlash* Rf system Installation Guide (part number 60-5233-385) and briefly describe the steps necessary to perform a separation run on the *CombiFlash* Rf 75 system. These quick start instructions assume that:

- the operator is familiar with the safety warnings in section 1 of the Installation Guide,
- the system has been installed according to section 3 of the Installation Guide,
- the system will be operated from the touch panel display.

Quick Start Instructions

1. Perform Thin Layer Chromatography (TLC) on the sample. From the TLC you will learn of a suitable column media and solvent system, and the difficulty of the compound separation.
2. Place a column bypass tube (P/N 209-0165-46) between the upper and lower column mounts.
3. Connect the Solvent Delivery Tube to the Injection Port located just above the upper column mount.
4. Place the Solvent A and B inlet lines into containers filled with the solvents chosen in step 1.

Note

If the selected solvents are not miscible with solvents from the last purification run, first prime the system with intermediate solvents.

5. Select the Tools>Auto Prime menu command to open a window from which you can prime the system. Touch the Play button. The system primes the internal tubing and then closes the Auto Prime window.

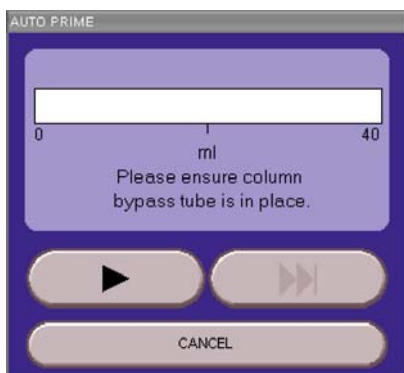


Figure 1: PeakTrak's Auto Prime window

6. Remove the column bypass tube.
7. With the column media identified by the TLC trials in step 1, select a column size appropriate for your intended sample load. Refer to the Help topic "RediSep Rf Column Selection Guide" for recommended sample load ranges.

If the separation is difficult ($\Delta R_f < 0.1$), choose a RediSep Rf Gold column and select the Gold Resolution mode. If using a standard RediSep Rf column for difficult separations, your sample load should be near the low end of the selected column's range.

8. Insert the selected column on the *CombiFlash* Rf 75 system. The system automatically loads an optimized method for that column. You can manually select the column media and size if the system does not detect the column.



Note

Raising the upper column mount assembly from its lowest point of travel triggers the column detection. If you exchange a column without lowering and raising the column mount, select the File>New menu command instead to detect the column.

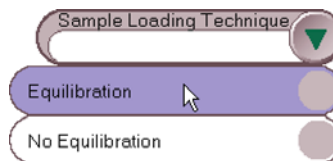
9. (Optional) Use the Solvent A and Solvent B controls to identify the solvents in post-run reports.



10. On the main window, touch the Play button.



11. From the Minimum Run Requirements window, select a Sample Loading Technique.



If the column should be equilibrated (recommended) before loading the sample, select the Equilibrate option and configure the Solvent delivery Tube as shown in Figure 2A.

Otherwise, select the No Equilibration option to skip column equilibration.

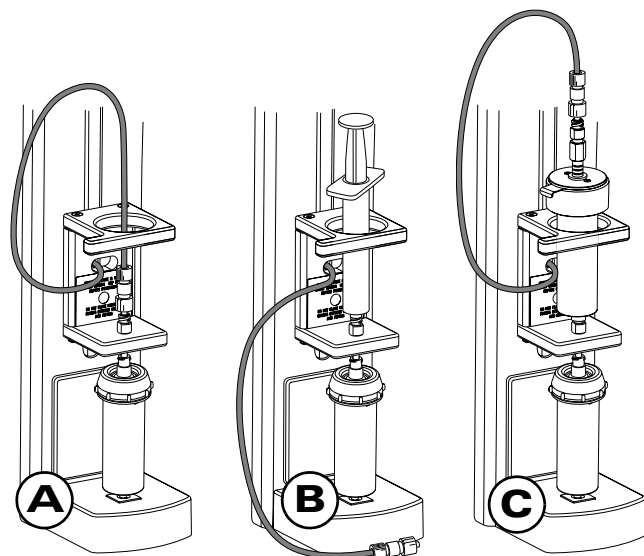


Figure 2: Solvent Delivery Tube Positions

A: Column equilibration, liquid sample separation
 B: Liquid sample injection
 C: Solid sample separation

12. If you selected No Equilibration, load the sample before touching the OK button. Refer to *Injecting Liquid Samples* or *Loading Solid Sample Cartridges* (on this page) for sample loading instructions.
13. Touch the OK button to start the run.
 - If you selected Equilibration, continue with step 14.
 - If you selected No Equilibration, skip steps 14 and 15 and continue with step 16.
14. The system will equilibrate the column and then pause for you to load the sample.
15. Load the solid or liquid sample as described in *Injecting Liquid Samples* or *Loading Solid Sample Cartridges*, then touch the OK button to continue.
16. Monitor the red absorbance trace.

The CombiFlash Rf system will proceed through the run and collect fluids in the fraction tubes. It isolates peak fluids from non-peak fluids. Tube change marks on the screen and the tube map help you locate your compounds.

Injecting Liquid Samples

Perform these sub-steps to load a liquid sample in steps 12 (for No Equilibration) or 15 (for Equilibration):

- a. Dissolve the sample in a minimal volume of Solvent A (or other miscible solvent) and draw the liquid into a syringe.
- b. Disconnect the Solvent Delivery Tube from the Injection Port.
- c. Inject the sample into the Injection Port (Figure 2B).
- d. Reconnect the Solvent Delivery Tube to the Injection Port (Figure 2A).

Loading Solid Sample Cartridges

Perform these sub-steps to load a solid sample in steps 12 (for No Equilibration) or 15 (for Equilibration):

- a. Prepare the solid sample cartridge according to the system's Help topic "Preparing Solid Samples," which describes how to use empty and pre-filled cartridges.
- b. Select a Solid Load Cartridge Cap that matches the size of the cartridge. The cap should have a female Luer adapter (P/N 209-0094-09) connected to its inlet. If not, attach one of the adapters from the CombiFlash Rf 75 accessory package.
- c. Press the lever on the side of the cap and fully extend the plunger.
- d. Slide the plunger into the cartridge until it reaches the top frit.
- e. Press the lever and push the cartridge into the cap. Align the cartridge so that it fits fully into the recess in the cap.
- f. Rotate the cartridge $\frac{1}{4}$ -turn to secure it in the cap. For the 5 and 25 gram cartridges, rotate the cartridge clockwise. For the 65 gram cartridge, rotate the cartridge counterclockwise.
- g. Disconnect the Solvent Delivery Tube from the Injection Port. and connect it to the Luer adapter on the cap (Figure 2C).
- h. Place the assembled cartridge and cap on the Injection Port.

Note

Refer to the instruction sheet titled "Adjustable Rf Solid Load Cartridge Cap for RediSep[®] Rf" (available at www.isco.com) for complete instructions.

Assistance Available

Should you need assistance with these instructions, please contact Teledyne Isco.

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