

CombiFlash NextGen Quick Start Guide

keywords: CombiFlash, NextGen, RediSep, solid load cartridge cap

Overview

These quick start instructions assume the following:

- The system has been installed according to Section 2 of the *CombiFlash Installation and Operation Guide* (the “user manual”) (PN 69-5253-032).
- The operator is familiar with the safety warnings in Section 1 of the user manual and in the *CombiFlash NextGen Important Information* document (PN 69-5253-086).
- The system will be operated from the touch screen display.

Quick Start Instructions

Focus Gradient Generator Workflow

The Focus Gradient Generator allows you to quickly create efficient preparative gradient methods using flash columns. This workflow assumes you will be performing at least two runs: first, a scouting run to determine optimized conditions and loading amount, followed by a focused preparative or series of preparative runs.

To begin, configure a column to use a scouting gradient.

1. Select a RediSep® column size appropriate for your sample amount. If the separation is difficult ($\Delta R_f < 0.2$), your sample loading should be near the low end of the selected column’s range.
2. Raise the column mount trolley from its lowest position, then insert the RediSep column on the CombiFlash NextGen system.
 - When the column is recognized, a dialog appears (Figure 1). Select **Scout for Focus Gradient**.

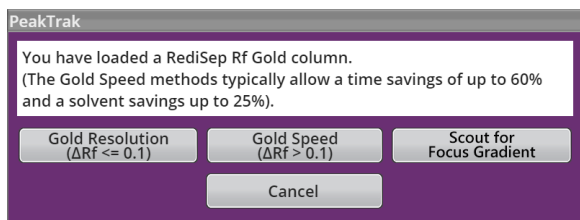


Figure 1. Gold Speed and Gold Resolution option upon RFID detection of RediSep Gold column.

- If the column is not automatically recognized by the RFID reader (or the system does not have this option), you can manually select the column media and size from the **Column** list on the **Main** screen (Figure 2). When selecting the column, also select the **Scout** method.
3. Choose a solvent system (Figure 2).

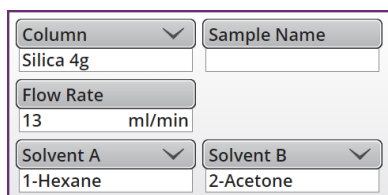


Figure 2. The Column selection list.

4. Open the **Method Editor**. Press a peak detection Details button to set **Detection Options** as required. Close the **Detection Options** window, then the **Method Editor**. The **Main** screen is revealed, showing the scouting gradient.
5. On the **Main** screen, press the **Start** button (▶). This opens the **Minimum Run Requirements** window.

6. From the **Minimum Run Requirement** window, choose a sample loading type. If the sample is soluble in the starting mobile phase and the sample will be injected, choose **Liquid**. Otherwise...
 - If the sample is not soluble in the starting mobile phase, use a solid load cartridge.
 - If the solid load cartridge is already prepared and loaded on the system, then choose **Solid**.
 - If the solid load cartridge has not been loaded on the system yet, then choose **Solid (Pause)**, which will equilibrate the column while you prepare the cartridge and will pause until you have installed the loaded cartridge. (**Solid (Pause)** is available only on NextGen systems with an injection valve.)

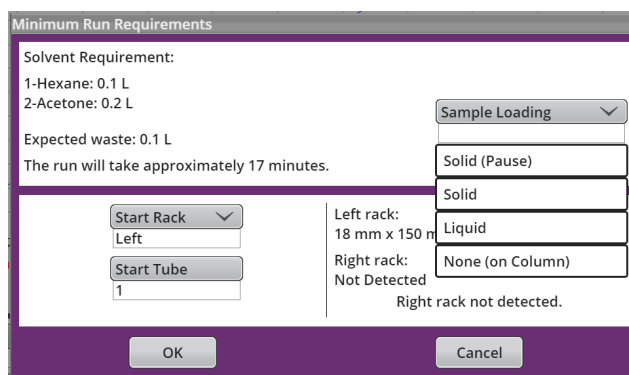


Figure 3. Minimum Run Requirement window and loading options.

7. Click **OK** to begin the run. If you selected...
 - **Liquid** or **Solid (Pause)** loading, continue to Step 8.
 - **Solid** loading, continue to Step 11.
8. The system will equilibrate the column. During this time, prepare the sample.
9. The NextGen will pause after performing the column equilibration. A dialog will either ask you to inject the sample or load the solid load cartridge.
10. Once the sample is loaded onto the system, click **OK** to begin the run.
11. As the run begins and progresses, you will be able to monitor the UV absorbance trace (or UV-Vis or other enabled detector options, depending on the system configuration). If needed, you could also modify or terminate the run:

After the scouting run is complete, its chromatogram is shown in an end-of-run window. Leave this window open: you will need it to generate a focused gradient method.

1. Press the **Focus Gradient** button just beneath the chromatogram. This window shows the peaks of the scouting run.
2. Select the peak at which you would like your purification to be optimized by using a finger on the touchscreen to move the vertical red peak indicator line.
3. Select the **Column for Focused Gradient** to use for the scaled-up preparative purification.
4. Choose the **Solid Load Amount**. Select either the amount of silica used in the solid load cartridge, or choose **Liquid Loading** if you are doing a liquid injection.
5. Press **Focus**. The system generates a focused gradient centered on the selected peak; this is loaded onto the **Main** screen.
6. On the **Main** screen, press the **Start** button (▶). This opens the **Minimum Run Requirements** window. Repeat Steps 5–11.

Traditional Workflow

The default method for a column or method development using thin layer chromatography (TLC) could also be used; however, running multiple TLC methods may take more time than using the Focus Gradient Generator.

1. Perform TLC on your sample to verify a suitable column media and solvent system and to determine the difficulty of the separation (ΔR_f of the compounds being separated).
2. Select a RediSep® column size appropriate for your sample amount. If the separation is difficult ($\Delta R_f < 0.2$), your sample loading should be near the low end of the selected column's range.
3. Raise the column mount trolley from its lowest position, then insert the RediSep column on the CombiFlash NextGen system.
 - If the column is not automatically recognized by the RFID reader (or the system does not have this option), you can manually select the column media and size from the **Column** list on the **Main** screen (Figure 4).

Figure 4. The Column selection list.

4. The system will load a universal method for the selected column size and media.
 - If using RediSep® Gold columns, you will be given the option of a **Gold Speed** or **Gold Resolution** method (Figure 5).

Figure 5. Gold Speed and Gold Resolution option upon RFID detection of RediSep Gold column.

- If you want to optimize the loaded default method, you can do so by selecting **Method Editor** from the **Main** screen menu.
5. Choose a solvent system (Figure 1).
 6. On the **Main** screen, press the **Start** button (▶). This opens the **Minimum Run Requirements** window.
 7. From the **Minimum Run Requirement** window, choose a sample loading type. If the sample is not soluble in the starting mobile phase, use a solid load cartridge. Otherwise...
 - If the solid load cartridge is already prepared and loaded on the system, then choose **Solid**.
 - If the solid load cartridge has not been loaded on the system yet, then choose **Solid (Pause)**, which will equilibrate the column while you prepare the cartridge and will pause until you have installed the loaded cartridge. (**Solid (Pause)** is available only on NextGen systems with an injection valve.)
 - If the sample is already loaded directly onto the column media, then select **None (on Column)**.

8. Click **OK** to begin the run. If you selected...
 - **Liquid or Solid (Pause)** loading, continue to Step 9.
 - **Solid** loading, continue to Step 12.
 - **None (on Column)**, equilibration will be skipped. Continue to Step 12.
9. The system will equilibrate the column. During this time, prepare the sample.
10. The NextGen will pause after performing the column equilibration. A dialog will either ask you to inject the sample or load the solid load cartridge.
11. Once the sample is loaded onto the system, click **OK** to begin the run.
12. As the run begins and progresses, you will be able to monitor the UV absorbance trace (or UV-Vis or other enabled detector options, depending on the system configuration). You also can also modify the run:
 - Modify the gradient without stopping the separation.
 - Change the fraction collection settings to **All, Peaks**, or **None**.
 - Open the **Method Editor** to change additional method parameters.
 - Insert an **Isocratic Hold**.
 - Advance to the next tube (**Tube Advance**).
 - **Terminate** the run.
13. Once the run is completed, an **End of Run** screen will appear, depending on whether or not you chose **End of Run Hold** earlier in the **Minimum Run Requirement** window.
 - If you chose **End of Run Hold**, a message will appear asking if you want to continue with air purge or extend your run (Figure 6).

Figure 6. Dialog at end of original method if **End of Run Hold** is enabled.

- If you did not choose **End of Run Hold**, the system will automatically wash the injection valve. The system will only air purge if a silica column is in place. It will not air purge any of the alternate media columns automatically.
14. As the system undergoes its valve wash and air purge processes, a report screen will appear. Here you can edit your report and save or print the pdf of the report (if enabled). Additionally, you have the option to use the completed method as modified for another separation or scale sized column.

CombiFlash NextGen Solid Load Cap Instructions

After inspecting the Solid Load Cartridge Cap (see Section 5.4.1 of user manual), ensure that the empty cartridge has a bottom frit. Then prepare the media and sample.

⚠ WARNING

Ensure that the solid load cartridge cap is installed properly and grounded. Refer to the *Adjustable Solid Load Cartridge Cap for RediSep Cartridges* instruction sheet (PN 69-5233-494) and the *Solid Load Cartridge Cap Grounding Clip Installation Instructions* (PN 60-5232-201).

To prepare an empty solid sample cartridge, follow these steps:

1. Dissolve your sample in a minimal amount of a suitable, volatile solvent.
2. Place the media into the solvent. If using silica gel, a particle size of 40–60 μm (240–400 mesh) is recommended. The amount of silica required is about four to five times the mass of your sample.
3. Agitate the solvent for a moment to allow the sample to adsorb to the silica.
4. Remove the solvent with a suitable method, such as rotary evaporation. Alternatively, you can dry the prepared cartridge after step 5 by drawing the solvent out using a vacuum or by using the NextGen system's air purge (if equipped).
5. Load the media and sample mixture into the cartridge. Tap the cartridge on the bench top to settle the mixture.
6. Place a frit on the top of the cartridge. Force the frit down against the mixture using a plunger suitable for the cartridge (a plunger is included with the solid load cartridge cap).
7. Wipe any residual powder inside the neck of the cartridge.

Note

You can remove solvent from a prepared cartridge by attaching the cartridge cap and manually purging the cartridge with air (Tools > Manual Control).

After you have prepared the pre-filled or empty cartridge, place the solid sample cartridge on the system:

8. Attach the desired adjustable cartridge cap:
 - 60-5237-047; fits 2.5 and 5 gram solid load cartridges.
 - 60-5237-048; fits 12 and 20 gram solid load cartridges.
 - 60-5237-044; fits 32 and 65 gram solid load cartridges.

9. Press the lever on the side of the cap and fully extend the plunger (Figure 7).

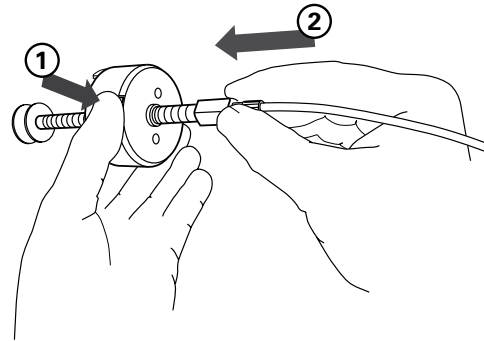


Figure 7. Press the lever and extend the plunger.

10. Slide the plunger into the cartridge until it reaches the top frit (Figure 8a).
11. Press the lever and push the cartridge into the cap. Align the cartridge so that it fits fully into the recess in the cap. (Figure 8b).
12. Load the solid sample cartridge with cap on the sample injection port.
13. Monitor the solid load cartridge (and RediSep column) for leaks while in use. If a solvent leak exists, stop the separation and correct the leak by either cleaning the sealing surfaces or by replacing the seal on the solid load cartridge.

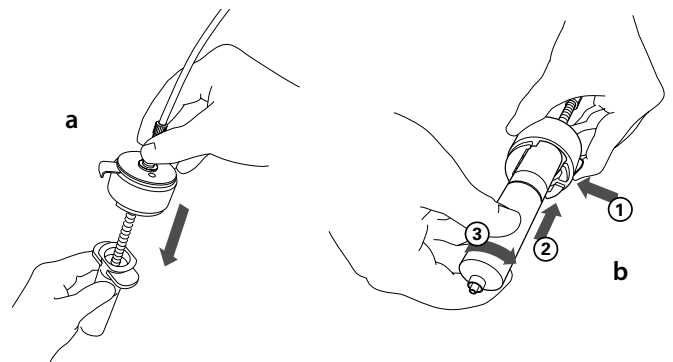


Figure 8. (a) Insert the plunger into the cap. (b) Align and push cartridge into the cap, then rotate the cartridge to secure it.