SepaFlash™ Columns

Simplify your purification, maximize your efficiency!

Introduction

Welcome to the SepaFlash™ Columns User Guide, designed to help you select and use the right flash chromatography column for your purification needs. SepaFlash™ columns from Santai Science are engineered to deliver high efficiency, reproducibility, and versatility, making them ideal for a wide range of applications, from routine separations to high-performance purifications.

This guide provides an overview of the different SepaFlash™ product lines, highlighting their key characteristics, recommended applications, and practical guidelines for optimal use. For complete specifications, including particle size, pore size, and pressure limits, please refer to the tables in this guide. However, always check the product label for the most accurate and detailed information specific to your column.

Let's get started with optimizing your chromatography workflow using SepaFlash™ Columns!

Standard Series
Ideal for general-purpose applications.

Large Size Series
Designed for high-capacity separations, meeting the needs of large-scale applications.

HP Series
High-performance options for demanding separations.

Functionalized Series
Tailored for specific sorbent requirements and optimized for separating both non-polar and polar samples.

iLOK™ & iLOK™-SL Series
Innovative pre-packed and solid-load columns featuring a patented locking design for convenient sample loading and handling.

SepaFlash™ Columns Compatibility

SepaFlash™ Columns are engineered for universal compatibility with a broad range of flash chromatography systems available on the market. Equipped with Luer-Lok® end fittings, these columns guarantee a secure and leak-free connection, ensuring seamless integration regardless of the equipment brand or model.

This universal design makes SepaFlash™ columns an ideal choice for laboratories with diverse chromatography systems, eliminating the need for specialized adapters or additional fittings. Whether you are using:

- Santai Science® SepaBean™
- Advion-Interchim® puriFlash®
- Biotage® Isolera® & Selekt®
- Buchi® Pure®
- Gilson® PLC®
- Grace® Reveleris®
- Teledyne Isco® CombiFlash®

Note: depending on the equipment used, an adapter may be required to use the SepaFlash™ Large Size Series. Please refer to the application note for further details.

BLL-0304: Luer connector kit for large columns (800 g, 1,600 g, 3 kg, and 5 kg)

BLL-NPT635-XXX: 10 kg cartridge adaptor available for 1/2, 3/8, 3/16, and 1/8 inches

SepaFlash™ Typical Column Characteristics

The tables below provide an overview of the key characteristics of our chromatography columns by product line.

SepaFlash™ Standard & Large Size Series

Column Size	Column ID x Length (mm)	Recommended Flow Rate (mL/min)					Maximum Pressure (psi / bar)
		Bare Irregular Silica Gels	Bare Spherical Silica Gels	All Aluminas	Functionalized Silicas		
					15 µm & 20 - 30/45 µm	40 - 63/75 µm	
4 g	12.3 x 97.5	15 - 40	15 - 30	10 - 30	Available in the SepaFlash™ SW (Spin-Welded) Column Series.		300 / 20.7
12 g	21.2 x 113.3	30 - 60	25 - 50	15 - 45			
25 g	21.2 x 163.1						
40 g	26.7 x 165.9	40 - 70	30 - 60	20 - 50			
80 g	30.9 x 242.5	50 - 100	40 - 80	30 - 70			250 / 17.2
120 g	37.4 x 254.3	60 - 150	45 - 90	40 - 80			
220 g	59.8 x 209.3	80 - 220	60 - 120	50 - 120			200 / 13.8
330 g	59.8 x 270.3						
800 g	78.2 x 382.9	100 - 300	200 - 300	100 - 200	40 - 80	50 - 100	100 / 6.9
1.6 kg	103.8 x 432.4	200 - 500		150 - 300			
3 kg	127.5 x 509.5		300 - 400				
5 kg	127.5 x 770.0		350 - 450				
10 kg	172.5 x 850.0	300 - 1,000	400 - 500	200 - 600			

SepaFlash™ SW (Spin-Welded) Column - HP & Functionalized Series

Column Size	Column ID x Length (mm)	Recommended Flow Rate (mL/min)				Maximum Pressure (psi / bar)
		Bare Silicas		Functionalized Silicas		
		25 µm, 50 µm & 20/25 - 45 µm	40 -75 µm	15 µm & 20 - 30/45 µm	40 - 63/75 µm	
4 g	12.4 x 113.8	15 - 30	15 - 40	5 - 15	10 - 20	400 / 27.5
12 g	21.4 x 134.8	20 - 50	30 - 60	10 - 25	15 - 30	
25 g	21.4 x 184.0					
40 g	26.7 x 184.4	30 - 60	40 - 70	15 - 30	20 - 40	
80 g	31.2 x 257.4	40 - 80	50 - 100	20 - 50	30 - 60	350 / 24.0
120 g	38.6 x 261.5	45 - 90	60 - 150	30 - 60	40 - 80	300 / 20.7
220 g	61.4 x 223.5	60 - 120	80 - 220	40 - 80	50 - 100	
330 g	61.4 x 280.2					250 / 17.2

SepaFlash™ Cartridges - iLOK™ & iLOK™-SL Series

Column Size	Column ID x Length (mm)	Sample Size (g)	Volume (mL)	Recommended Flow Rate (mL/min)	Maximum Pressure (psi / bar)
Small Size Formats					
4 g	12.8 x 60	0.004 - 0.400	8	15 - 40	200 / 13.8
12 g	21.8 x 76	0.012 - 1.200	27	30 - 60	
25 g	21.6 x 126	0.025 - 2.500	46		
40 g	26.8 x 125	0.040 - 4.000	70	40 - 70	
60 g	36.6 x 99	0.060 - 6.000	104	60 - 150	
80 g	31.2 x 193	0.080 - 8.000	147	50 - 100	
100 g	60.4 x 61	0.100 - 10.000	176	80 - 220	150 / 10.3
120 g	36.6 x 204	0.120 - 12.000	215	60 - 150	200 / 13.8
220 g	60.6 x 131	0.220 - 22.000	377	80 - 220	150 / 10.3
330 g	60.6 x 187	0.330 - 33.000	539		
Large-Size Formats (sample size and flow rate are provided for reference and may require adjustments.)					
800 g	127 x 140	0.8 - 80.0	1,395	200 - 400	100 / 6.9
1.6 kg	127 x 250	1.6 - 160.0	2,760		
3 kg	127 x 440	3.0 - 300.0	5,165	200 - 500	
5 kg	127 x 692	5.0 - 500.0	8,610		
7 kg	127 x 1,000	7.0 - 700.0	12,510		

Using the SepaFlash™ Sapphire Series: A Step-by-Step Guide

Cartridge Installation

- Securely attach the cartridge to your flash chromatography system, ensuring a proper fit with the connectors.
- Once the cartridge is installed, refer to the system's user guide for detailed setup and operation instructions.
- Alternatively, you can consult this quick reference guide to assist with your purification process.

Note: Always store unused columns in a dry place at room temperature with the end caps securely in place. Remove the end caps only when installing the column.

Sample Loading

The solubility of the compound determines whether to use liquid or dry loading when introducing the sample onto the SepaFlash™ column.

- **Liquid loading** involves dissolving the sample in the minimum amount of the weakest possible solvent. The dissolved sample is then added to the top of the SepaFlash™ column or introduced via the sample injector using a syringe.

- **Dry loading** is recommended when the sample has limited solubility in weak solvents. In this case, a stronger solvent is used to fully dissolve the sample, which is then pre-adsorbed onto a small quantity of sorbent. After evaporating the solvent using a rotary evaporator, the sample-sorbent mixture is loaded into an empty SepaFlash™ iLOK™ empty solid-load cartridge (see procedure below), which is placed on top of the flash column or directly on the 15 % free space of the SepaFlash™ iLOK™-SL openable column.

How to Use the SepaFlash™ iLOK™ Cartridges

Step 1:
Unscrew and remove the cap from the iLOK™ empty column tube, remove the frit, and place the column tube on a support stand.

Step 2:
Fill the column tube with silica gel mixed with the sample (for solid-load), the adsorbent (for chromatographic column) or with 85 % of silica (for combined application as shown on the picture). Tap gently to settle and avoid overfilling.

Step 3:
Place the frit on the top of the column tube.

Step 4:
Use the insertion tool to press the frit into the column tube, ensuring the frit is as straight as possible.

Complete the next two steps only for the combined application. For solid-load use, proceed directly to Step 7.

Step 5:
Add the silica gel mixed with the sample to the top of the column (blue section), gently tap to settle and place the second frit on the column tube.

Step 6:
Use the insertion tool to press the frit into the column tube, ensuring the frit is as straight as possible.

Step 7:
Finally, securely fasten the column by fully tightening it with the cap screwing closing tool.

Ensure the cap is fully tightened before use!

Step 8:
Your SepaFlash™ iLOK™ cartridge is now ready for use. It is recommended toperform a pre-equilibration step with 3 - 5 column volumes before loading your sample.

SepaFlash™ iLOK™-SL

Each SepaFlash™ iLOK™-SL cartridge features a built-in inserter for convenient liquid injection with a syringe. For solid loading, remove the inserter and follow steps 5 to 8 as outlined above, replacing the frit with the dispersion unit at step 6.

Typical Recommended Column Conditions

The conditions outlined in this table serve as a general starting point for column use and maintenance. However, specific applications may require adjustments based on factors such as sample properties, solvent compatibility, and system requirements. It is recommended to optimize these parameters as needed to achieve the best chromatographic performance and column longevity.

Steps	Normal Phase Mode	Reversed Phase Mode	HILIC Phase Mode
Pre-conditionling	Flush the column with at least 3 CV using hexanes or the initial mobile phase conditions.	Flush the column with at least 3 CV of ≥ 90 % organic solvent (typically methanol or acetonitrile) to activate the phase.	Flush the column with at least 5 CV of ≥ 90 % organic solvent.
Equilibration	Flush the column with at least 3 CV using the initial mobile phase conditions. **Typical solvent:** hexane & ethyl acetate mixture.	Flush the column with at least 2 CV using the initial mobile phase conditions. **Typical solvent:** organic solvent (typically methanol or acetonitrile) in water.	Flush the column with at least 10 CV using the initial mobile phase conditions. **Typical solvent:** 60 - 95 % acetonitrile with aqueous buffers like ammonium acetate or formate.
Disclamer	**SepaFlash™ columns are single-use, but customers may validate an FDA-compliant cleaning process at their own risk. Santai Science provides no warranty for reuse, as it may affect separation efficiency and fraction purity.**		
General Guidelines for Cleaning	1. **Prevent drying:** after the first use, do not allow the column to dry out and ensure the air purge on the instrument is turned off. 2. **Use intermediate solvents when necessary:** if the run solvents are immiscible with the storage solvents, rinse the column with an intermediate solvent. 3. **Remove organic solvents:** flush the column with at least 3 CV of the solutions listed under "Cleaning" to remove organic modifiers or strong organic solvents.		
Cleaning	Rinse the column with at least 3 CV of 100 % of the most polar solvent in the elution mixture, optionally in reverse flow. If needed, rinse with 5 CV of 100 % isopropanol. Do not use 100 % methanol; instead, use a dichloromethane:methanol mixture (≤ 25 % of methanol).	If buffers or additives were used, rinse the column with 5 CV of 95:5 water:organic solvent (skip this cleaning step if not required), followed by 5 CV of 95:5 organic solvent:water (typically methanol or acetonitrile in water).	Rinse the column with 10 CV of 95:5 water:organic solvent, then 10 CV of 95:5 organic solvent:water (e.g., methanol or acetonitrile). If needed, clean with 10 CV of 50 mM ammonium formate or acetate (50:50 aqueous:acetonitrile).
General Guidelines for Storage	**To ensure proper storage, fill the column with the recommended solvents listed under "Storage Conditions" and keep it in a dry place at room temperature with the end caps securely in place.**		
Storage Conditions	Store the column in 100 % isopropanol.	Store the column in ≥ 70 % organic solvent (typically methanol or acetonitrile in water).	Store the column in 80 % acetonitrile in water or 100 % isopropanol.

Notes: CV stands for "Column Volume," which refers to the total internal volume of the column. Before reusing the column, ensure proper re-equilibration by running it in normal flow mode with the initial solvent conditions before the next injection.

Optimizing Column Conditions

If the typical recommended conditions do not provide optimal performance, consider one of these adjustments:

- **For difficult separations,** use gradient elution, starting with a solvent where Rf ≤ 0.2 and gradually increasing polarity. If resolution remains poor, consider a two-step purification, using a second column for finer separation.
- **For acid-sensitive compounds,** to prevent degradation, use neutral or amine-modified silica. Alternatively, deactivate silica by flushing the column with 5 - 10 CV of a solvent containing 1 - 3 % triethylamine (TEA) before running the separation.
- **Consider column stacking** by connecting two identical pre-packed columns in series to improve resolution and loading capacity. Adjust flow rate to manage back pressure.
- **During pre-conditioning step,** increase equilibration time if retention is inconsistent. Ensure solvent compatibility and adjust buffer pH for better peak shape.

Feel free to contact our technical support team at support@santaisci.com if you need help or advice.