

Biotage® Sfär Flash Columns

User Guide



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Connecting Biotage® Sfär Columns to Biotage® Selekt and Isolera™

By connecting the tubing, the columns are easily attached to all Biotage® flash systems. Ensure you remove the Luer-slip adapter to make a secure fitting with the outlet tubing. When needed, keep the Luer-slip adapter on to fit other systems.



Equilibration

Equilibration of flash columns prior to sample addition

- » Removes entrapped air
- » Eliminates the heat band effect from solvent adsorption on a dry column from the separation run
- » Ensures equilibrium between the solvent and the silica.

The minimum recommended equilibration volume is two CV for Biotage® Sfär columns.

Caution: Always equilibrate the column with the standard insert (below) in place to minimize expansion of the o-ring inside the column cap.



Reversed-Phase Equilibration

Reversed-phase chromatography columns require several steps to ensure they become wet with solvent. Wetting reversed-phase media improves compound retention and therefore loading capacity. For optimal purification with Biotage® Sfär C18 D and Biotage® Sfär Bio family columns, follow these steps.

1. Flush with 100% for 3-5 column volumes (CV) of the strong (organic) solvent, typically methanol, acetonitrile or THF.
2. Flush another 3 to 5 CV with 50% strong + 50% aqueous (if system compatible additives are required, include them also at this stage).
3. Flush with another 3 to 5 CV with the initial gradient conditions of your run.

Pressure Safety

Ensure to use the correct pressure settings when running the columns. The columns have been designed to withstand prolonged running at maximum pressure but should never be exposed to any higher pressures as there might be cracks or burst of the plastic that might cause personal injuries.

Static Electricity

When using Biotage flash columns or any other commercial or homemade column, care must be taken to avoid the consequences of static electricity buildup. This is particularly important when using the systems with flammable, non-conducting solvents operating under high-flow conditions. Static electricity can be generated as non-polar liquids flow through columns, tubing, pipes, valves, and filters during chromatographic operations. It can also be brought to the operating environment by human bodies that have accumulated static charge; please follow your local regulations that apply to static electricity hazards. Please ensure you follow the safety precautions related to your chromatography system.

Sample Loading

Note: Sample solvent choice will have an impact on purification. Samples should be dissolved at the highest concentration in the weakest solvent. Use of a strong, polar solvent will compromise purification by reducing load and purity.

1. Liquid Loading Through the Luer Port with Syringe

To load a sample, remove plunger from a syringe. Attach the empty syringe to the Biotage® Sfär inlet Luer. Add sample to the empty syringe. When instructed by the instrument software, insert the plunger and inject the sample. Allow pressure to stabilize (solvent/air movement in outlet tubing stops). Remove the syringe.

2. Liquid Loading Direct-on-frit

Remove the cap from the Biotage® Sfär 'Duo' column. Press the center of the insert sideways. Remove the insert from the column. Add sample to the frit using a pipette or direct pour-on. Attach the insert to the cap and screw the cap back on to the column. Note: when using a 200 g or 350 g column it is recommended to use the supplied wrench (available separately) to remove and attach the cap.

3. Dry Loading

Note: Each Biotage® Sfär column from the 'Duo' product range is supplied with an insert, which allows liquid injection via a syringe. If internal dry loading is to be used, this insert must be removed.

Pre-packed Samplet® cartridges allow purification of liquid samples in a dry form, eliminating solvent impact. Samplet® cartridges are inserted into the Biotage® Sfär 'Duo' columns and should have the dissolution solvent evaporated prior to

insertion by air or vacuum oven (see Solvent Vapor Pressure table on p. 5). Pre-packed Samplet® cartridges are available in the following media types:

Part Number	Description
SAS-0445-0010	Biotage® Sfär Silica Samplet® for 5/10 g Column
SAS-0445-0025	Biotage® Sfär Silica Samplet® for 25 g Column
SAS-0445-0100	Biotage® Sfär Silica Samplet® for 50/100 g Column
SAS-0445-0350	Biotage® Sfär Silica Samplet® for 200/350 g Column
SAS-0825-0010	Biotage® Sfär HMN-R Samplet® for 5/10 g Column
SAS-0825-0025	Biotage® Sfär HMN-R Samplet® for 25 g Column
SAS-0825-0100	Biotage® Sfär HMN-R Samplet® for 50/100 g Column
SAS-0825-0350	Biotage® Sfär HMN-R Samplet® for 200/350 g Column
SAS-0401-0010	Biotage® Sfär C18 Samplet® for 5/10 g Column
SAS-0401-0025	Biotage® Sfär C18 Samplet® for 25 g Column
SAS-0401-0100	Biotage® Sfär C18 Samplet® for 50/100 g Column
SAS-0401-0350	Biotage® Sfär C18 Samplet® for 200/350 g Column
SAS-0909-0010	Biotage® Sfär KP-Amino Samplet® for 5/10 g Column
SAS-0909-0025	Biotage® Sfär KP-Amino Samplet® for 25 g Column
SAS-0909-0100	Biotage® Sfär KP-Amino Samplet® for 50/100 g Column
SAS-0909-0350	Biotage® Sfär KP-Amino Samplet® for 200/350 g Column

Table 1. Samplet® options for Biotage® Sfär columns.

To load a sample, remove the cap from the Biotage® Sfär column. Press the center of the insert sideways. Remove the insert from the column. Add sample to the samplet and let it dry (see Figure 1.c below). Insert the Samplet® into the orange column cap, as shown below, ensuring that the Samplet fits firmly into the column cap. Now place the column cap (with Samplet) onto the column and screw together firmly. Ensure the cap is even and fully tightened down. Screw the cap back on the column.

Note: when using a 200 g or 350 g column it is recommended to use the supplied wrench (available separately) to remove and attach the cap.

4. Bulk Loading

A dried, pre-adsorbed sample on a media can also be added directly to the top on the column. Specific media amounts are

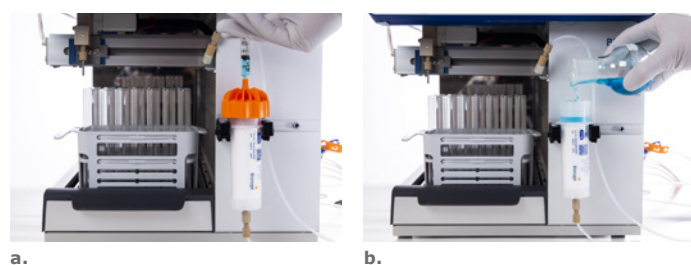


Figure 1. Biotage® Sfär loading techniques.

- Liquid loading through the Luer port with a syringe.
- Liquid loading direct-on-frit.
- Dry loading using a Samplet®.
- Bulk loading.

required (Table 5). To Load a sample, remove the cap from the Biotage® Sfär column. Press the center of the insert sideways. Remove the insert from the column. Add the correct amount of dried media with sample to the top of the column. Ensure it is evenly distributed. Screw the cap back on to the column.

Note: when using a 200 g or 350 g column it is recommended to use the supplied wrench (available separately) to remove and attach the cap.

5. External Loading

Dried, pre-adsorbed sample on a media can also be used, utilizing the Biotage® Sfär DLV (dry load vessel) modules.

Three DLV plungers exist for development scale purifications:

- » DVL-010 – can be used with up to 10g of silica
- » DLV-025 – can be used with up to 25 g of silica
- » DLV-050 – can be used with up to 100g of silica

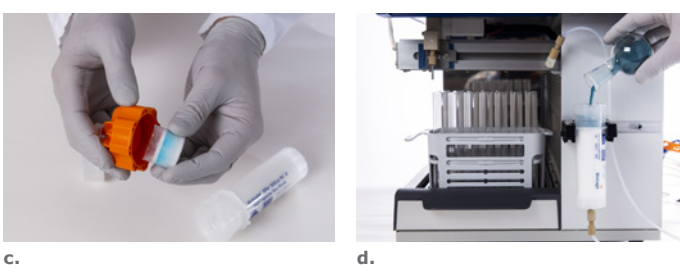
They attach directly to the top of the Biotage® Sfär column and uses a Luer-Lok to secure good operation. DLV columns exist in both empty and pre-packed versions.

Part Number	Description
DLV-0445-0010	Biotage® Sfär Silica DLV Column with 5 g Silica
DLV-0445-0025	Biotage® Sfär Silica DLV Column with 10 g Silica
DLV-0445-0050	Biotage® Sfär Silica DLV Column with 25 g Silica
DLV-0825-0010	Biotage® Sfär HMN-R DLV Column with 5 g HMN-R
DLV-0825-0025	Biotage® Sfär HMN-R DLV Column with 10 g HMN-R
DLV-0825-0050	Biotage® Sfär HMN-R DLV Column with 25 g HMN-R
DLV-0000-0010	Biotage® Sfär DLV Empty 10 g Column w. frit
DLV-0000-0025	Biotage® Sfär DLV Empty 25 g Column w. frit
DLV-0000-0050	Biotage® Sfär DLV Empty 50 g Column w. frit
DLV-0000-0100	Biotage® Sfär DLV Empty 100 g Column w. frit

Table 2. Dry load vessel columns for Biotage® Sfär flash columns.

Waste Handling

Biotage flash columns consist of polypropylene plastic, polyethylene plastic, silicon rubber and pure silica. Ensure that you follow the local guidelines for hazardous waste for proper disposal.



Drying Procedures for Samplet® Cartridges

Make sure samples will not degrade on silica or at selected temperature.



Solvent Vapor Pressure (VP)	Vacuum Pressure	Heat
< 100 mm Hg	Moderate to high (650 mm Hg to 550 mm Hg)	With heat. Set the temperature 15–20 °C below the solvent boiling point.
100–300 mm Hg	Low to moderate (700 mm Hg to 600 mm Hg)	Without heat
> 300 mm Hg	None to low (760 mm Hg to 650 mm Hg)	Without heat

Table 3. Vacuum pressures for various solvent vapor pressures.

Samplet® Size (g)	Solvent Vapor Pressure > 100 mm Hg	Solvent Vapor Pressure < 100 mm Hg
1 (for 5/10 g Column)	20 min	30 min
3 (for 25 g Column)	25 min	35 min
10 (for 50/100 g Column)	30 min	45 min
35 (for 200/350 g Column)	45 min	60 min

Table 4. Drying time.

Volumes and Weight for Bulk Dry Load Media


Make sure samples will not degrade on silica or at selected temperature.

Biotage® Sfär Column Size	Usable Volume (mL)	Silica Mass (g)
5 g	3	1,5
10 g	3	1,5
25 g	14	7
50 g	28	14
100 g	28	14
200 g	115	57
350 g	115	57

Table 5. Volume and weight for bulk dry load media.



Declaration of Conformity



Declaration of Conformity

Biotage Sweden AB, Box 8, SE-751 03 Uppsala, Sweden

declare under our sole responsibility that the

Columns: Biotage® Sfär
Part number: FSXX-YYYY-ZZZZ

XX=RD(Silica D), RS(Silica), UD(Silica HC D), US(Silica HC), UD(C18 D), BD(Bio C4 D/ Bio C18 D), AD(Amino D)


YYYY=0445(Duo 60µm and 60µm), 0443(High Capacity Duo 20µm and High Capacity 20µm), 0401(Duo 100 Å 30 µm), 0411(Duo 300 Å 20 µm), 0412(Duo 300 Å 20 µm), 0454(Duo 50 µm)

ZZZZ=0005(5g), 0006(6g), 0010(10g), 0012(12g), 0025(25g), 0030(30g), 0050(50g), 0060(60g), 0100(100g), 0120(120g), 0200(200g), 0240(240g), 0350(350g), 0400(400g)

to which this declaration relates are in conformity with the following directives, standards and other normative documents;

Directive 2014/68/EU **Pressure Equipment Directive (PED)**
Standard used: EN/IEC 61010-1:2010 (3rd Edition)

Uppsala, 24 September 2018



 (Steve Jordan, Chief Scientific Officer - Biotage)

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Biotage® Sfär columns Declaration of Conformity.

Biotage® Sfär Product Specifications

Part Number	Media Type	Column Type	Model	Weight (g)	Silica Size (µm)
FSRD-0445-0005	Silica	Duo - Samplet® Support	Silica D	5	60
FSRD-0445-0010	Silica	Duo - Samplet® Support	Silica D	10	60
FSRD-0445-0025	Silica	Duo - Samplet® Support	Silica D	25	60
FSRD-0445-0050	Silica	Duo - Samplet® Support	Silica D	50	60
FSRD-0445-0100	Silica	Duo - Samplet® Support	Silica D	100	60
FSRD-0445-0200	Silica	Duo - Samplet® Support	Silica D	200	60
FSRD-0445-0350	Silica	Duo - Samplet® Support	Silica D	350	60
FSRS-0445-0005	Silica	Sealed	Silica	5	60
FSRS-0445-0010	Silica	Sealed	Silica	10	60
FSRS-0445-0025	Silica	Sealed	Silica	25	60
FSRS-0445-0050	Silica	Sealed	Silica	50	60
FSRS-0445-0100	Silica	Sealed	Silica	100	60
FSRS-0445-0200	Silica	Sealed	Silica	200	60
FSRS-0445-0350	Silica	Sealed	Silica	350	60
FSUD-0443-0005	Silica	Duo - Samplet® Support	Silica HC D	5	20
FSUD-0443-0010	Silica	Duo - Samplet® Support	Silica HC D	10	20
FSUD-0443-0025	Silica	Duo - Samplet® Support	Silica HC D	25	20
FSUD-0443-0050	Silica	Duo - Samplet® Support	Silica HC D	50	20
FSUD-0443-0100	Silica	Duo - Samplet® Support	Silica HC D	100	20
FSUD-0443-0200	Silica	Duo - Samplet® Support	Silica HC D	200	20
FSUD-0443-0350	Silica	Duo - Samplet® Support	Silica HC D	350	20
FSUS-0443-0005	Silica	Sealed	Silica HC	5	20
FSUS-0443-0010	Silica	Sealed	Silica HC	10	20
FSUS-0443-0025	Silica	Sealed	Silica HC	25	20
FSUS-0443-0050	Silica	Sealed	Silica HC	50	20
FSUS-0443-0100	Silica	Sealed	Silica HC	100	20
FSUS-0443-0200	Silica	Sealed	Silica HC	200	20
FSUS-0443-0350	Silica	Sealed	Silica HC	350	20
FSUD-0401-0006	Silica C18	Duo - Samplet® Support	C18 D	6	30
FSUD-0401-0012	Silica C18	Duo - Samplet® Support	C18 D	12	30
FSUD-0401-0030	Silica C18	Duo - Samplet® Support	C18 D	30	30
FSUD-0401-0060	Silica C18	Duo - Samplet® Support	C18 D	60	30
FSUD-0401-0120	Silica C18	Duo - Samplet® Support	C18 D	120	30
FSUD-0401-0240	Silica C18	Duo - Samplet® Support	C18 D	240	30
FSUD-0401-0400	Silica C18	Duo - Samplet® Support	C18 D	400	30
FSBD-0411-0010	Silica C18	Duo - Samplet® Support	Bio C18 D	10	20
FSBD-0411-0025	Silica C18	Duo - Samplet® Support	Bio C18 D	25	20
FSBD-0411-0050	Silica C18	Duo - Samplet® Support	Bio C18 D	50	20
FSBD-0411-0100	Silica C18	Duo - Samplet® Support	Bio C18 D	100	20
FSBD-0412-0010	Silica C4	Duo - Samplet® Support	Bio C4 D	10	20
FSBD-0412-0025	Silica C4	Duo - Samplet® Support	Bio C4 D	25	20
FSBD-0412-0050	Silica C4	Duo - Samplet® Support	Bio C4 D	50	20
FSBD-0412-0100	Silica C4	Duo - Samplet® Support	Bio C4 D	100	20
FSAD-0909-0005	Silica NH2	Duo - Samplet® Support	KP-Amino D	5	50
FSAD-0909-0011	Silica NH2	Duo - Samplet® Support	KP-Amino D	11	50
FSAD-0909-0028	Silica NH2	Duo - Samplet® Support	KP-Amino D	28	50
FSAD-0909-0055	Silica NH2	Duo - Samplet® Support	KP-Amino D	55	50
FSAD-0909-0110	Silica NH2	Duo - Samplet® Support	KP-Amino D	110	50
FSAD-0909-0220	Silica NH2	Duo - Samplet® Support	KP-Amino D	220	50
FSAD-0909-0380	Silica NH2	Duo - Samplet® Support	KP-Amino D	380	50

Table 6. Biotage® Sfär column specifications.

Pore Width (Å)	Capacity	Flow Rate (mL/min.)	CV (mL)	Pressure (bar)	Pressure (psi)	Label Color	Pack size
60	50-500 mg	18	9	17	300		20
60	100-1000 mg	40	15	17	300		20
60	200-2000 mg	80	42	17	300		20
60	0.5-5 g	120	80	17	300		10
60	1-10 g	120	150	17	300		10
60	2-20 g	200	310	12	175		4
60	3.5-35 g	200	530	12	175		4
60	50-500 mg	18	9	20	300		20
60	100-1000 mg	40	15	20	300		20
60	200-2000 mg	80	42	20	300		20
60	0.5-5 g	120	80	20	300		10
60	1-10 g	120	150	20	300		10
60	2-20 g	200	310	12	175		4
60	3.5-35 g	200	530	12	175		4
50	100-1000 mg	18	9	17	250		20
50	200-2000 mg	40	15	17	250		20
50	0.5-5 g	80	42	17	250		20
50	1-10 g	120	80	17	250		10
50	2-20 g	120	150	17	250		10
50	3.5-35 g	200	310	12	175		4
50	7.0-70 g	200	530	12	175		4
50	100-1000 mg	18	9	20	250		20
50	200-2000 mg	40	15	20	250		20
50	0.5-5 g	80	42	20	250		20
50	1-10 g	120	80	20	250		10
50	2-20 g	120	150	20	250		10
50	3.5-35 g	200	310	12	175		4
50	7.0-70 g	200	530	12	175		4
100	50-500 mg	6	9	17	250		2
100	100-1000 mg	12	17	17	250		2
100	200-2000 mg	25	45	17	250		2
100	0.5-5 g	50	85	17	250		2
100	1-10 g	50	164	17	250		2
100	2-20 g	75	328	12	175		1
100	3.5-35 g	100	582	12	175		1
300		25	15	17	250		2
300		40	41	17	250		2
300		70	78	17	250		2
300		70	151	17	250		2
300		25	15	17	250		2
300		40	41	17	250		2
300		70	78	17	250		2
300		70	151	17	250		2
60		6	9	17	250		20
60		12	15	17	250		20
60		25	33	17	250		20
60		50	66	17	250		10
60		50	132	17	250		10
60		100	264	12	175		4
60		100	510	12	175		4

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