Biotage® Flash 75/150

User Manual

Biotage®

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Introduction

Biotage® Flash 75 and Biotage® Flash 150 are flash purification systems based on prepacked cartridge technology that provide users of traditional self-packed glass columns with three critical operational benefits: speed, safety, and performance.

Speed

Unlike glass columns, which operate at low pressure and have very poor flow distribution, Biotage flash cartridges are optimized for high speed separations. Purifications that might otherwise have used up half a working day can be run in 15 to 45 minutes. For a chemist running just a single reaction and separation per day, the annual gain in productivity can be as high as 15 additional weeks each year; time that could be used to develop new compounds or processes. With the rising cost of labor, the efficiencies afforded by this alone can exceed the cost of the flash system and a year's supply of cartridges.

Safety

Laboratory and process safety is a growing concern with many companies carefully reviewing current procedures and techniques to reduce the risk of chemical exposure, injury, and consequently their liability. Biotage Flash systems eliminate the concerns of using glassware under pressure by utilizing durable polyethylene cartridges that are prepacked and completely self-contained, eliminating user exposure to silica dust, HP-API, or any contaminants left after a separation.

Performance

In addition to speed and safety, Biotage flash cartridges improve both the performance and the reproducibility of separations. The use of Biotage patented radial compression technology (see Figure 1) reduces interstitial spaces (void volume) within the packed bed. The optimized bed density means that compounds are collected in narrow bands, which results in higher separation efficiency. Unlike glass columns, each Biotage flash cartridge will perform in the same way, time after time.

Flash 75/150 systems include everything you need to separate compounds in a fume hood with standard laboratory mounting bars, clamps, and an inert gas such as nitrogen. A typical Flash 75/150 system can be assembled in minutes and be ready to run samples in less than an hour.

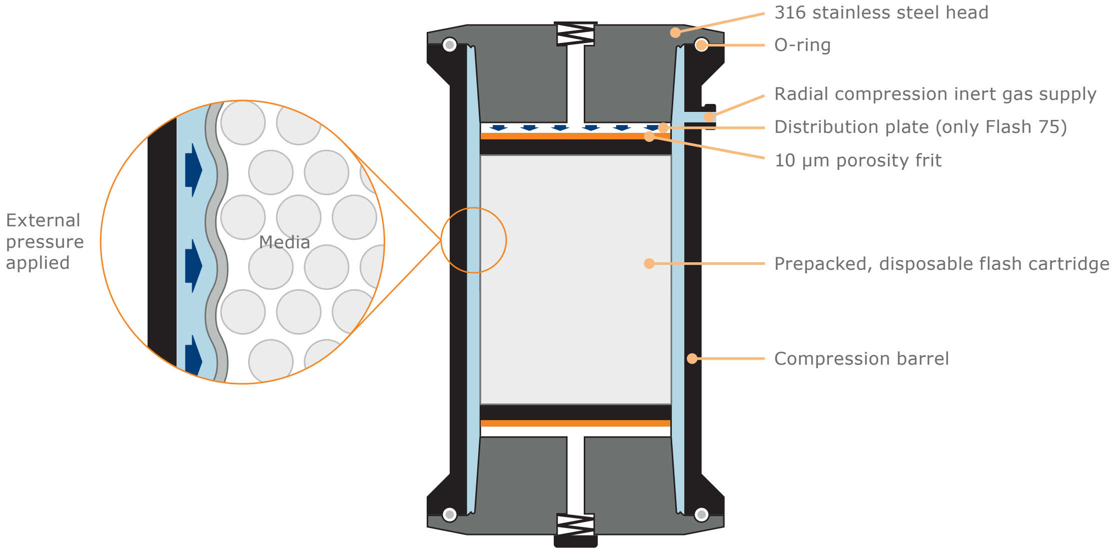

Figure 1. Biotage patented radial compression technology improves flash separation performance and increases separation speed.

System Components

Prepacked Biotage® Flash Cartridge

The prepacked flash cartridge from Biotage is the key to the radial compression module's improved performance. Flash cartridges are available in a wide variety of sizes for optimum separation of samples ranging from 50 mg to 8 kg. Biotage offers Flash 75 and Flash 150 cartridges and radial compression modules for samples up to 1000 grams; see Table 1 below.*

Each flash cartridge is prepacked as standard with normal phase silica, C18-bonded silica, amine-functionalized silica, or activated carbon, and is individually wrapped with a water repelling plastic film to minimize water vapor adsorption. Each case of cartridges includes certificates of compliance and conformance with detailed information for your records in GLP or cGMP environments.

The flash cartridge itself is made from FDA compliant, medium density polyethylene. The flow distribution plate and 10 µm porosity frits are also fabricated from FDA compliant polyethylene.

* This user manual deals specifically with the Flash 75 and 150 cartridge series. For information on additional cartridge diameters, contact Biotage. See contact information on the back of this document or visit our website www.biotage.com.

Radial Compression Module

The radial compression module (see Figure 3), together with the flash cartridge, is the heart of the system. It is an anodized aluminum barrel with an instant-tight fitting for connection to the inert gas outlet on the AM-190 manifold (see page 3).

The safety features on the barrel include a safety relief valve that activates at pressures in excess of 110 psi (7.6 bar) for Flash 75 and 150 psi (10.3 bar) for Flash 150, and a red pressure indicator that sticks out when there is more than 20 psi (1.4 bar) of inert gas pressure inside the radial compression module (see Figure 2).

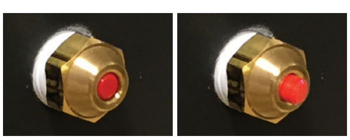

Figure 2. The pressure indicator sticks out when the pressure inside the radial compression module is more than 20 psi (1.4 bar).

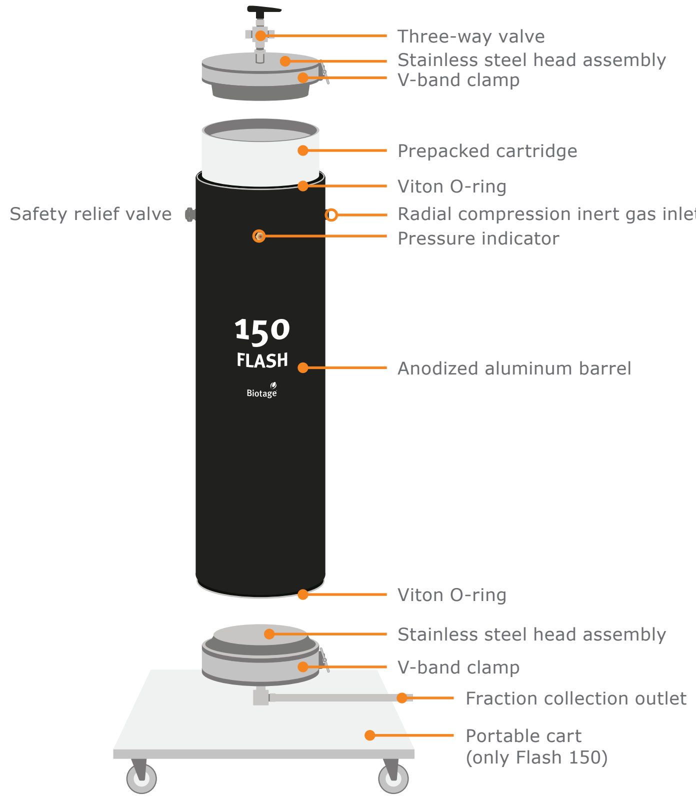

Figure 3. The components of the Flash 150 radial compression module. The Flash 75 module has the same features but instead of being delivered with a portable cart it has mounting pads on the side of the barrel for easy connection to lab scaffolding or support bars.

A Viton O-ring sits inside a groove in the top lip of the barrel and seals the radial compression module against leakage of gas. The bottom opening of the barrel has an identical groove and O-ring. Two replacement O-rings are provided with the start-up kit (see page 5).

The Flash 150 radial compression module comes with a portable cart with casters. The Flash 75 barrel is easily attached to standard ½" (12.7 mm) Flex-Frame type laboratory mounting bars using a pair of standard mounting rods that screw into the mounting pads on the side of the barrel. With the rods in place, the module can be secured with standard mounting clamps (not included).

Specification	Flash 75M	Flash 75L	Flash 150M	Flash 150L
Size D x H	75 x 150 mm (3" x 6")	75 x 300 mm (3" x 12")	150 x 300 mm (6" x 12")	150 x 600 mm (6" x 24")
Mass of Biotage® KP-Sil Silica (approx.)	0.4 kg	0.8 kg	2.5 kg	5.0 kg
Column Volume (approx.)	0.5 liter	1.0 liter	4.3 liters	8.6 liters
Sample Size	Up to 80 grams	Up to 160 grams	Up to 500 grams	Up to 1000 grams

Table 1. The specifications of the Flash 75 and Flash 150 cartridges.

Head Assemblies

Two stainless steel head assemblies, with knife-edges for sealing the cartridge, attach to the top and bottom of the barrel by means of V-band clamps, which can be easily hand-tightened to seal the flash cartridge inside the barrel. The top head assembly is fitted with a three-way valve that connects to the solvent reservoir and the outlet of the Biotage sample injection module (Biotage® SIM). The bottom head assembly has a tube compression fitting; this is the fraction collection outlet of the radial compression module.

AM-190 Manifold

The AM-190 manifold (see Figure 4) regulates the radial compression pressure and the flow of sample and solvent into the radial compression module. The manifold is easily attached to standard fume hood mounting bars using the two mounting rods supplied with the manifold; see “Set Up the AM-190 Manifold” on page 9.

The AM-190 manifold comes equipped with the following features. See the corresponding letters in Figure 4.

A. An inlet for pressurized inert gas, with an instant-tight fitting for attaching tubing.

B. A safety relief valve that activates at pressures in excess of 150 psi (10.3 bar).

C. A locking pressure regulator (labeled **PRR-01**), with a water trap (C2), for increasing or decreasing the inert gas pressure to the radial compression module.

D. A pressure gauge (labeled **PG-01**) for monitoring the inert gas pressure delivered to the radial compression module.

E. A valve (labeled **MV-01**) for turning the flow of compressed inert gas to the radial compression module on or off (see Table 2), with an instant-tight fitting for attaching tubing (E2).

F. A locking pressure regulator (labeled **PRR-02**) for increasing or decreasing the inert gas pressure to the solvent reservoir and sample injection module (SIM).

G. A pressure gauge (labeled **PG-02**) for monitoring the inert gas pressure delivered to the solvent reservoir and SIM.

H. A valve (labeled **MV-03**) for turning the flow of compressed inert gas to the SIM on or off (see Table 2), with an instant-tight fitting for attaching tubing (H2).

I. A valve (labeled **MV-02**) for turning the flow of compressed inert gas to the solvent reservoir on or off (see Table 2), with an instant-tight fitting for attaching tubing (I2).

Valve Positions

Valve	Off/Vent	On/Pressurize
MV-01		
MV-02 and MV-03		

Table 2. The positions for the three valves on the AM-190 manifold.

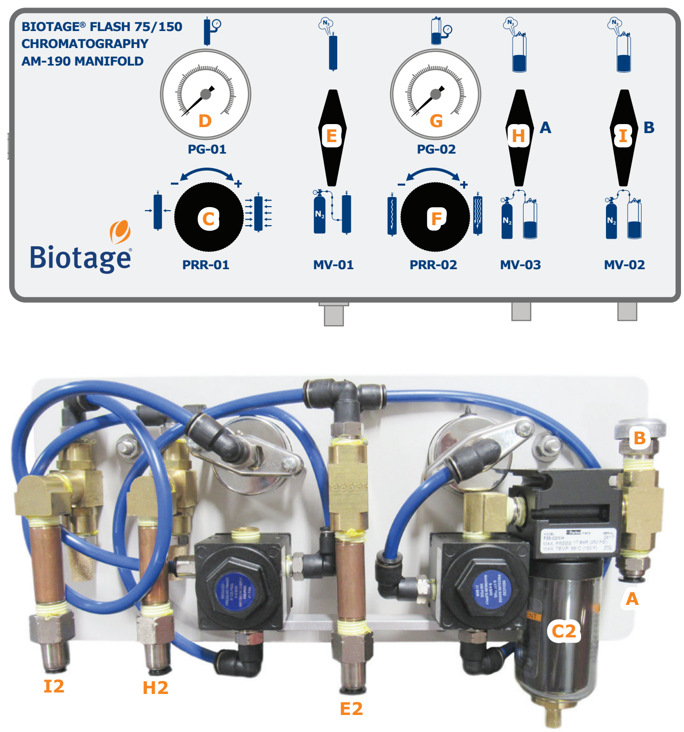

Figure 4. The AM-190 manifold (bottom) regulates the flow of inert gas into the radial compression module, solvent reservoir, and sample injection module (SIM). The front panel of the AM-190 manifold (top) offers easy-to-use switches to set all flows.

A = pressurized inert gas inlet, B = safety relief valve, C = regulator for the gas pressure to the radial compression module, C2 = water trap, D = gauge for the pressure delivered to the radial compression module, E = compress/vent valve for the radial compression pressure, E2 = instant-tight fitting for connecting a gas tube between the manifold and the radial compression module, F = regulator for the gas pressure to the solvent reservoir and sample injection module (SIM), G = gauge for the inert gas pressure delivered to the solvent reservoir and SIM, H = compress/vent valve for the solvent pressure, H2 = instant-tight fitting for connecting a gas tube between the manifold and the solvent reservoir, I = compress/vent valve for the SIM pressure, and I2 = instant-tight fitting for connecting a gas tube between the manifold and the SIM.

Solvent Reservoir

Biotage provides an electropolished 316 stainless-steel solvent reservoir, available in a 12-liter capacity for the Flash 75 system and 37-liter and 60-liter capacities for the Flash 150 system. The solvent reservoir is fitted with an instant-tight fitting for pressurized inert gas, an on/off valve for solvent flow, and a safety relief valve that activates at pressures in excess of 109 psi (7.5 bar).

The oval lid of the reservoir is fitted with a clamp that screws shut, and has a solvent proof, PTFE-encapsulated Viton O-ring to seal the lid in place. See "Set Up the Solvent Reservoir" on page 10 for safety precautions on how to secure the lid once the reservoir has been filled with solvent.

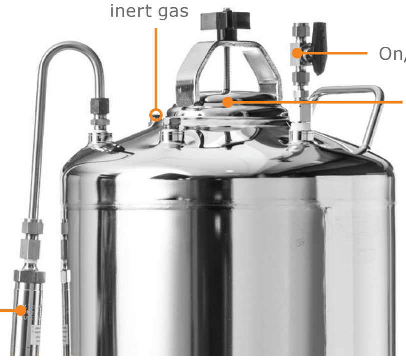

Figure 5. The 60-liter solvent reservoir with the two-way valve open.

Sample Injection Module (Biotage® SIM) Kit

Biotage provides a sample injection module (SIM) kit, available in 500 mL, 1000 mL, and 2000 mL sizes, to simplify the handling of routine samples as well as viscous oils and samples with poor solubility that need to be adsorbed directly onto silica. SIM 500 is optimized for Flash 75M and 75L, SIM 1000 for Flash 150M, and SIM 2000 for Flash 150L.

Illustrated in Figure 6, the SIM body is constructed of a welded 316 stainless steel tube with a sanitary clamp at the top and a stainless steel 1/8" (Flash 75) or 1/4" (Flash 150) tube fitting at the bottom. The top lid comes with a three-way valve. One port supplies inert gas to drive liquid sample onto the cartridge. The other port supplies solvent through the SIM when using a pre-adsorbed sample loading technique.

The SIM is supplied with six sanitary gaskets (two of each type, see "Sanitary Gaskets" below), one chain clamp (P/N 01520), 25 top frits (P/N FTF-0225), 25 bottom frits with sealing rings (P/N FBS-1025), and one frit insertion tool (P/N 01596 for SIM 500 and 1000 and P/N 03339 for SIM 2000).

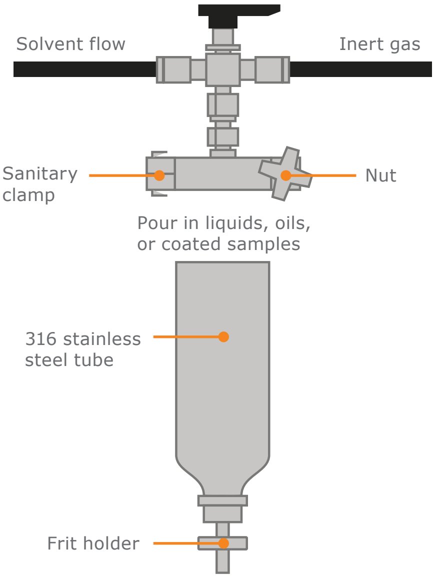

Figure 6. The sample injection module (SIM) simplifies the handling of routine samples as well as viscous oils and samples with poor solubility.

Sanitary Gaskets

The SIM includes three different types of sanitary gaskets; PTFE/EPDM, Viton, and EPDM (see Figure 7). Biotage recommends the black and gray PTFE/EPDM gasket for most applications, as it is resistant to most normal phase solvents and mixtures. The gasket is moderately soft and seats when the sanitary clamp is firmly tightened. The PTFE/EPDM gasket may leak a small amount of solvent initially if the SIM is completely full of liquid.

The black Viton and EPDM gaskets are very compressible and will seal reliably up to 100 psi (6.9 bar), even with a finger-tight sanitary clamp. EPDM, which has a green color dot on the edge, is resistant to polar solvents but will swell in many common aliphatic and aromatic solvents. Viton, which has yellow and white color dots on the edge, is resistant to non-polar solvents, but will swell in the typical polar modifiers. For solvent compatibility information, see Table 3 below.

Solvent	PTFE/EPDM	Viton	EPDM
Hexane	FAIR	GOOD	POOR
Toluene	GOOD	GOOD	POOR
Ethyl acetate	GOOD	POOR	GOOD
Dichloromethane	FAIR	FAIR	FAIR
Methanol	GOOD	POOR	GOOD

Table 3. Solvent compatibility.

Table 3 shows a limited number of commonly used solvents. To check the compatibility of a particular solvent or solvent mixture, soak a gasket in the solvent/mixture for one hour and then inspect it for swelling. If the gasket swells in use, allow it to dry out in a fume hood until it returns to its normal size (typically overnight). Perform a periodic check of your gaskets and replace a gasket that shows sign of wear or damage or that leaks at your typical operating pressures.

Figure 7. There are three sanitary gaskets for the SIM: 1) the black and gray PTFE/EPDM gasket (P/N 415891SP), 2) the black Viton gasket with yellow and white color dots on the edge (P/N 01615), and 3) the black EPDM gasket with green dots on the edge (P/N 01616).

Grounding Kit

The system can build up high static charges as non-polar solvents flow through PTFE tubing, membrane filters, and/or across a bed of dry silica. To eliminate any risk of a static discharge, the systems and ancillary containers must be grounded before use.

The pre-wired grounding kit that is supplied with the system includes a complete wiring harness assembly (see Figure 8) and a pair of fraction collection assemblies (a smaller one for the Flash 75 system and a larger one for the Flash 150 system, see Figure 9). For instructions on how to install the grounding kit, see "Ground the System" on page 12.

Figure 8. The wiring harness assembly included in the grounding kit.

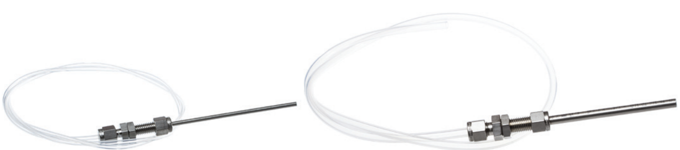

Figure 9. The fraction collection assembly for the Flash 75 system (left) and the Flash 150 system (right).

Start-Up Kit

Biotage provides a start-up kit consisting of the following items:

- For supplying inert gas, the kit includes four (4) six-foot lengths (1830 mm) of polyethylene tubing, 1/4" O.D., 0.04" wall thickness, color-coded in BLUE (P/N 01487), YELLOW (P/N 01354), GREEN (P/N 00547), and RED (P/N 00546).
- For supplying solvent, the kit includes:
  - Flash 75: one (1) three-foot (914 mm) and one (1) one-foot length (305 mm) of 1/8" O.D. CLEAR PTFE tubing (P/N 00088), and one (1) six-foot length (1830 mm) of 1/4" O.D. CLEAR PTFE tubing (P/N 00089).
  - Flash 150: one (1) twelve-foot length (3658 mm) of 1/4" O.D. CLEAR PTFE tubing (P/N 00089).
- Two (2) spare O-rings for the radial compression module (P/N 00778 for Flash 75 and P/N 01420 for Flash 150).
- One (1) two-part inert gas supply fitting, a 3/8" NPT reducing bushing with a 1/4" (M) NPT attached to an instant-tight tubing connector (P/N 03072).
- One (1) three-way valve for the radial compression module (P/N 04562).
- One (1) grounding kit (P/N FGD-15075).
- One (1) cartridge extraction tool (P/N 02131 for Flash 75 and P/N 01428 for Flash 150).
- Two (2) disposable 10 mL syringes (P/N 01656).
- One (1) Luer injection port (P/N02838). Only Flash 150.
- One (1) user manual (P/N 02140).

For detailed information on how tubing and fittings are attached and assembled, refer to the section "Installation" on page 9.

Cartridge Extraction Tool

A cartridge extraction tool is provided to simplify the removal of used flash cartridges from the barrel of the radial compression module. The tool consists of a machined stainless steel rod with a handle on one end and two swinging, bladed "leaves" on the other (see Figure 10). A sliding ring on the shaft locks the leaves into a position perpendicular to the shaft of the tool.

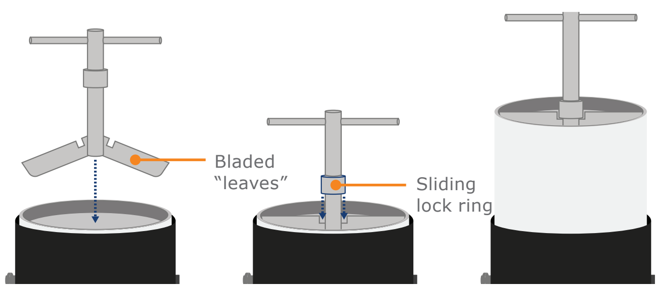

Figure 10. The cartridge extraction tool simplifies the removal of a used cartridge from the radial compression module.

To use the tool, slip the lock ring to the bottom of the shaft so that the leaves are held in place. Hold the tool by the handle and push the tool firmly into the top of the cartridge. The bladed surface of the leaves will "bite" into the plastic edge of the cartridge, allowing it to be easily pulled out. Moving the sliding lock ring up will release the leaves so the tool can be removed.

Chemical Resistance

The following chemicals can be used in the wetted parts of the system:

- Acetone
- Dichloromethane (methylene chloride, DCM)
- Toluene
- Acetonitrile (MeCN)
- Tetrahydrofuran (THF)
- Triethylamine (TEA)
- N,N-dimethylformamide (DMF)
- Ethyl acetate (ethyl ethanoate, EtOAc)
- N-methyl-2-pyrrolidone (NMP)
- Dimethylsulfoxide (DMSO)
- Ethanol (EtOH)
- Methanol (MeOH)
- 2-propanol (isopropanol, IPA)
- Formic acid (methanoic acid, HCOOH)
- Acetic acid (Ethanoic acid, HOAc, AcOH)
- Deionized water (H_2O)
- Trifluoroacetic acid (TFA), max 5 % (by volume)
- n-Heptane
- Piperidine (pip)
- Pyridine (pyr)
- Ammonia (NH_3) conc. 23–27 % (by weight)
- tert-Butyl methyl ether (TBME)
- Diethyl ether
- 10 mM phosphate buffered saline, pH= 7.4
- 50 mM phosphate-citrate buffer, pH= 5.0
- Boric acid/NaOH/KCl buffer, pH=11

Note: The sample injection module (SIM) is delivered with three types of sanitary gasket. Always check the gaskets' compatibility with the solvent or solvent mixture that you want to use. For more information, see “Sanitary Gaskets” on page 4.

Safety

Intended Use

Biotage Flash 75 and Flash 150 systems are intended solely for purification. The systems must be operated in a laboratory environment with an ambient temperature between 4°C and 32°C (39°F and 90°F) by trained professionals. The system must not be installed or used near a potential ignition source. It is the responsibility of the customer to classify (zone) their particular environment in order to verify that it meets the requirements of the directive 1999/92/EC. All operations must be performed:

- According to the user documentation delivered with the system.
- According to instructions available at www.biotage.com.
- According to instructions given by the technical support staff from Biotage.
- Within limits set by the system's technical specification and in line with user standard operating procedures (SOPs).

Failure to follow those instructions and operate within the limits set by the technical specification may result in personal injury and/or equipment damage.

Education, Training, and Competence

It is your responsibility to provide all applicable health and safety regulations to your personnel. You must also ensure that all personnel involved in the operation and maintenance of the system fulfill the following criteria:

- Have the necessary education, training, and competence required for the intended use of the system.
- Observe general and specific safety regulations for the use of the system and its accessories at all times in order to reduce the risk of personal injury, fire, and explosion.

Warranty and Liability

See the "Biotage Terms & Conditions of Sale" document at www.biotage.com.

Service

All service must be performed by an authorized Biotage service engineer. Before handing over the system for service, it should be emptied of all liquid and cleaned from harmful residues.

It is the responsibility of the customer to inform Biotage® 1-Point Support™ representatives if the system has been used with hazardous biological, radioactive, or toxic samples and/or solvents, prior to any service being performed.

If returning equipment to Biotage, this should be done in accordance with the material return procedures supplied separately by Biotage.

Only genuine Biotage spare parts must be used in the system.

Labels

Labels used on the system:

0036

In accordance with all the essential requirements of all applicable European product directives; see the Declaration of Conformity.

In accordance with the ATEX Product Directive, 2014/34/EU for Group II, Category 2G equipment. The equipment is intended for use in areas in which explosive atmospheres caused by gases, vapors, or mists or air/dust mixtures are likely to occur.

Manufacturer.

Consult accompanying user documentation.

Note: The system is ASME rated and in accordance with both U.S. and Canadian safety standards.

Safety Requirements

You must observe all safety requirements when installing and operating the system. Failure to install or use the system in a manner specified by Biotage may result in personal injury and/or equipment damage.

If the system has been damaged or does not function properly, turn off the inert gas supply, depressurize the radial compression module and the solvent reservoir, and contact Biotage 1-Point Support (www.biotage.com).

Installation

- The system must be unpacked and installed as described on page 9.
- Follow regional safety practices when handling and moving shipping boxes and containers, and when moving the system.
- The total weight of the package including the system is between 16 and 84 kg (35 and 185 lbs) depending on the system configuration. Use suitable lifting equipment when moving the package.

- The system must be placed in a well-ventilated fume hood or an equivalent enclosure that is capable of exhausting 2.5 m^3/min (90 ft^3/min). Follow local and national safety regulations for installing a system inside a fume hood and the safety regulations supplied by the fume hood manufacturer.
- Never install or use the system near a potential ignition source.
- The system, when operated with non-polar solvents such as hexane, methylene chloride, etc., can build up a high static electricity charge, which in certain conditions can be dangerous. To eliminate any risk of a static discharge, the systems and ancillary containers must be grounded before use as described on page 12. Failure to follow these grounding instructions may result in equipment damage, personal injury, or death.
- A trained person must verify that the system and ancillary containers are grounded before each run. Nominal resistance must be below 5 Ohm between ground and each metal point in the system.
- Always connect inert gas such as nitrogen to the gas inlet on the AM-190 manifold.
- Ensure that the inert gas tubing connected to the system cannot come in contact with chemicals. Corrosives and solvents can dissolve the tubing.
- The AM-190 manifold must be mounted horizontally so that the water trap can function correctly.
- The outlets of the three safety relief valves (located on the radial compression module, AM-190 manifold, and solvent reservoir) must be directed away from the operator.
- Ensure that the tubes are not longer than necessary for your setup so that there is no risk of stepping or tripping over them.
- To avoid injury to yourself or damage to the system, set the inert gas supply pressure to between 100 and 125 psi (6.9 to 8.6 bar).
- Do not over-tighten the fittings or the tubing may become damaged.
- Never modify the system components in any way and always use spare parts supplied by Biotage.

Operation

- Use the system only for its intended purpose, as described in the user documentation delivered with the system and user documentation available at www.biotage.com. If the system is used in a manner not specified by Biotage, the safety features of the system may be compromised.
- The system must not be operated unattended.
- Never operate the system if damaged.
- The system is operated using pressurized inert gas. Ensure that all seals are properly mounted and that all clamps are properly closed before introducing pressurized gas into the system.
- Confirm that the upper V-band clamp is tight before compressing the radial compression module. Only hand-tighten the clamp, to a maximum torque of 11.3 Nm (100 lbf-in). If the cartridge is not sealed properly, replace the O-ring and retry, or contact Biotage 1-Point Support.
- Inspect the V-band clamps before and after each use. The clamp must be replaced when it shows any signs of wear and tear, or at 500 pressure cycles, whichever comes first. Only use clamps and screws supplied by Biotage.
- Never loosen the V-band clamps when the radial compression module is under pressure. A red pressure indicator on the radial compression module sticks out when the pressure is above 20 psi (1.4 bar); see Figure 2 on page 2.
- Never remove a tube or system component when it is under pressure.
- Reduce the pressure using the solvent pressure regulator (labeled **PRR-02**) to less than 50 psi (3.5 bar) before blowing down the Flash 150 radial compression module with inert gas.
- When closing the solvent reservoir lid, ensure that it is seated properly to ensure a secure seal.
- Never run the system without a cartridge, or with the wrong size of cartridge.
- Always equilibrate the cartridge.
- Equilibrate the cartridge at a low flow rate to avoid extreme temperature rise.
- Only use waste/fraction collection vessels that are grounded or made of glass.
- If the system is placed in a walk-in fume hood, a fume extractor must be used for open waste/fraction collection vessels.
- Follow all applicable safety procedures when working with bottled gas.
- Follow all generally-accepted lab safety procedures and applicable laws and regulations.
- Always follow local and national safety regulations related to storage, handling and disposal of chemicals, samples and waste.
- Read and understand the safety data sheet (SDS) provided by the chemical manufacturer before storing, handling, working with, or disposing of any chemical or hazardous substance.
- Personnel working with or near the system must wear applicable safety clothing and gear (such as solvent-resistant clothing and gloves, steel toe shoes, and face and eye protection) that comply with local and national safety regulations.

Installation

Warning

- Follow regional safety practices when handling and moving shipping boxes and containers, and moving the system.
- The system must be placed in a well-ventilated fume hood or an equivalent enclosure that is capable of exhausting 2.5 m^3/min (90 ft^3/min). Follow local and national safety regulations for installing a system inside a fume hood and the safety regulations supplied by the fume hood manufacturer.
- Never install or use the system near a potential ignition source.
- If the system is placed in a walk-in fume hood, a fume extractor must be used for open waste/fraction collection vessels.
- The total weight of the package including the system is between 16 and 84 kg (35 and 185 lbs) depending on the system configuration. Use suitable lifting equipment when moving the package.

Note: We recommend that the boxes and packing materials are retained in case the system needs to be returned for service or moved to another location. If you need to ship the system, please contact Biotage 1-Point Support for instructions.

Set Up the Radial Compression Module

Warning

- The outlet of the safety relief valve must be directed away from the operator.

1. Remove the assembled radial compression module (see D in Figure 13 on page 11) from the shipping container.
2. Attach the three-way valve supplied in the start-up kit onto the top head assembly using a 9/16" wrench.
3. Determine the best installation location for the radial compression module. Consider the following:
   - The outlet of the safety relief valve (see Figure 3 on page 2) must be directed away from the operator.
   - There must be sufficient space above the radial compression module to insert and remove cartridges.
4. If using a Flash 75 radial compression module:
   a. Screw the two mounting rods into the mounting pads located on the side of the barrel.
   b. Attach the barrel to the fume hood's mounting bars using mounting clamps (not supplied).
5. If using a Flash 150 radial compression module, roll the cart with the module into a walk-in fume hood.

Set Up the AM-190 Manifold

Warning

- The AM-190 manifold must be mounted horizontally so that the water trap can function correctly.
- The outlet of the safety relief valve must be directed away from the operator.

1. Remove the AM-190 manifold (see A in Figure 13 on page 11) from the shipping container.
2. Attach the two mounting bars to the manifold using a 5/32" Allen key.
3. Determine the best mounting location for the manifold. Consider the following:
   - Choose a location that allows you to easily access both the manifold and the radial compression module.
   - The outlet of the safety relief valve (see B in Figure 4 on page 3) must be directed away from the operator.
4. Attach the AM-190 manifold to the fume hood's mounting bars using mounting clamps. Ensure that the manifold is mounted horizontally.

Set Up the Sample Injection Module (Biotage® SIM)

Note that the use of the sample injection module (SIM) is optional.

1. Remove the SIM (see B in Figure 13 on page 11) from the shipping container.
2. Open the bag of bottom frits and push one plastic frit into a sealing ring. Save the other frits and sealing rings for use later.
3. Unscrew the lower half of the stainless steel frit holder and drop the sealing ring and frit into the lower half; see Figure 22 on page 16.

   Note: Ensure that the plastic frit is at the bottom of the frit holder.
4. Screw the lower half of the frit holder back in place. Finger-tight is normally adequate.
5. Determine the best mounting location for the SIM. Consider the following:
   - Typically, the SIM should be mounted so that the bottom tube fitting is 1" to 3" (25 to 76 mm) above the radial compression module.
   - Ensure to leave enough space to insert and remove cartridges from the radial compression module.
   - The SIM should be in a convenient location for pouring in liquid or solid sample.
6. Attach the SIM to the fume hood's mounting bars using the chain clamp provided.

Set Up the Solvent Reservoir

Warning

» The outlet of the safety relief valve must be directed away from the operator.

» When closing the solvent reservoir lid, ensure that it is seated properly to ensure a secure seal.

1. Remove the solvent reservoir (see C in Figure 13 on page 11) from the shipping container.
2. If the two-way on/off valve is not installed:
   a. Remove the on/off valve from the inside of the reservoir.
   b. Install the on/off valve into the 1/4" NPT fitting on the solvent reservoir. Turn the valve clockwise by hand. Use a wrench (not provided) on the hex section of the adapter to fully tighten it into the solvent reservoir.
   c. Install the lid on the solvent reservoir by tilting and lowering it into the mouth of the reservoir. Hand-tighten the clamp to secure the lid and ensure that the lid is seated properly to ensure a secure seal.
3. Place the solvent reservoir in a convenient spot on the benchtop or floor of the fume hood. Ensure that you leave enough space for your fraction collection vessels and that the outlet of the safety relief valve (see Figure 5 on page 4) is directed away from the operator.

Attach the Inert Gas Supply and Solvent Lines

Warning

» Always connect inert gas such as nitrogen to the gas inlet on the AM-190 manifold.

» Follow all applicable safety procedures when working with bottled gas.

» Set the inert gas supply pressure to between 100 and 125 psi (6.9 to 8.6 bar).

» Ensure that the inert gas tubing connected to the system cannot come in contact with chemicals. Corrosives and solvents can dissolve the tubing.

» Ensure that the tubes are not longer than necessary for your setup so that there is no risk of stepping or tripping over them.

» Do not over-tighten the fittings or the tubing may become damaged.

The start-up kit includes all of the tubing necessary for operation (see page 5).

1. Ensure that:
   a. The inert gas supply is turned off.
   b. The two-way valve on the solvent reservoir is closed.
   c. The three valves on the AM-190 manifold are set to their off/vent positions (see Table 2 on page 3)
2. Install a pressure regulator set to a maximum of 125 psi (8.6 bar) to your inert gas supply.
3. Install the brass fitting supplied with the start-up kit (the two-part assembly with a 3/8" NPT reducing bushing and a 1/4" NPT instant-tight tubing connector):
   » If using an inert gas supply in the fume hood, remove the standard hose barb and replace it with the brass fitting.
   » If using bottled inert gas, remove the 3/8" NPT reducing bushing and then thread the 1/4" instant-tight tubing connector directly onto your pressure regulator.
4. Connect all the tubing, except the fraction collection tube, as shown in the left schematic in Figure 13 or, if not using a SIM, as shown in Figure 12. The fraction collection tube is supplied with the grounding kit and is connected during the grounding of the system; see the instructions on page 12.

   Cut the tubing so that they are long enough for your setup. The system has both instant-tight fittings and fittings with nuts and ferrules, see the instructions below. Test the seal by pulling on the tubing.

   Note: All tubing should be cut with square ends using a tube cutter. Do not use scissors as they tend to flatten the tubing and leave a rough edge.

   Note: If using a SIM, the clear PTFE tubing attached to the solvent reservoir must be long enough to reach both to the radial compression module (for when equilibrating and loading liquid samples, see the left schematic in Figure 13) and the SIM (for when loading solid samples, see the right schematic in Figure 13).

   Note: The Flash 75 start-up kit contains both 1/4" and 1/8" clear PTFE tubing. The 1/8" tubing is to be used between the SIM and the radial compression module.

Fitting with Nut and Ferrules

1. Slide the nut and ferrules onto the tube as shown in Figure 11.
2. Push the tube into the fitting until it seats and then finger-tighten the nut. Use a 7/16" or 9/16" wrench to turn the nut 1 to 1¼ turn past finger-tight (the first time, after that less is required). Do not over-tighten.
3. Test the seal by gently pulling on the tube.

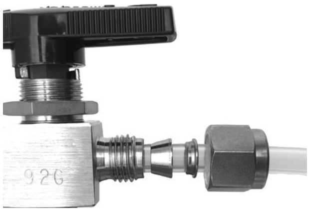

Figure 11. How to slide the nut and ferrules onto the tubing.

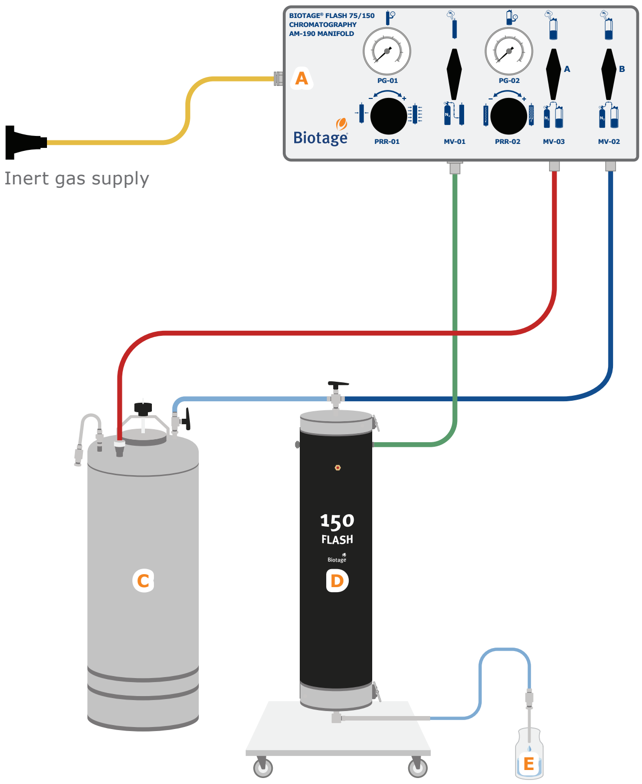

Figure 12. Schematics of a system without the sample injection module (SIM).

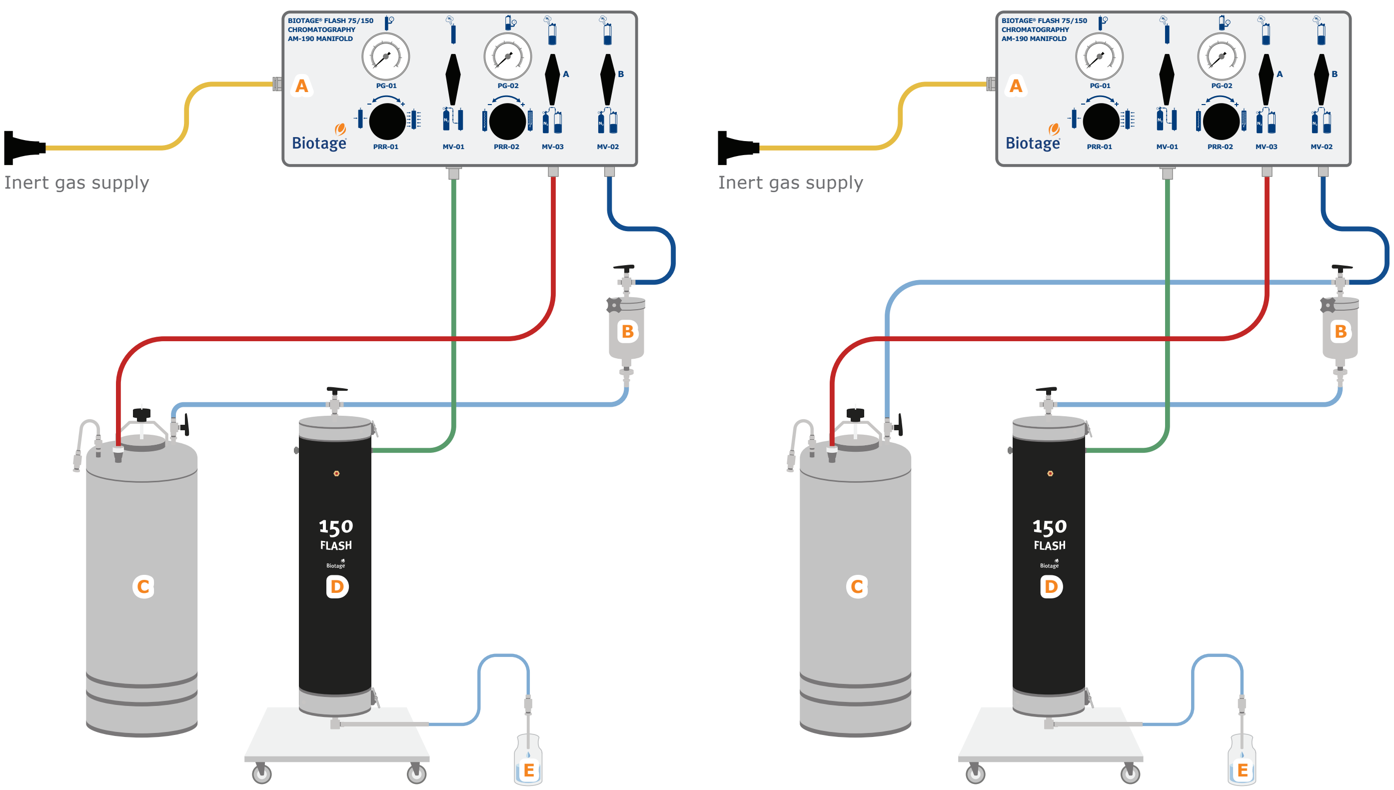

Figure 13. Schematics of a system ready-for-use when equilibrating the system and loading liquid sample (left) and when loading solid sample (right).
A = AM-190 manifold, B = sample injection module (SIM), C = solvent reservoir, D = radial compression module, and E = fraction collection vessel.
Yellow tube = pressurized inert gas inlet, green tube = inert gas to the radial compression module, red tube = inert gas to the solvent reservoir,
blue tube = inert gas to the sample injection module, and clear PTFE tubes (light blue in the schematics) = solvent supply, SIM outlet, and fraction collection.

Ground the System

Warning

- The system, when operated with non-polar solvents such as hexane, methylene chloride, etc., can build up a high static electricity charge, which in certain conditions can be dangerous. To eliminate any risk of a static discharge, the systems and ancillary containers must be grounded before use as described below. Failure to follow these grounding instructions may result in equipment damage, personal injury, or death.
- A trained person must verify that the system and ancillary containers are grounded before each run. Nominal resistance must be below 5 Ohm between ground and each metal point in the system.

The pre-wired grounding kit that is supplied with the system includes a complete wiring harness assembly (see Figure 14) and a pair of fraction collection assemblies (see Figure 15).

Figure 14. The wiring harness assembly included in the grounding kit.

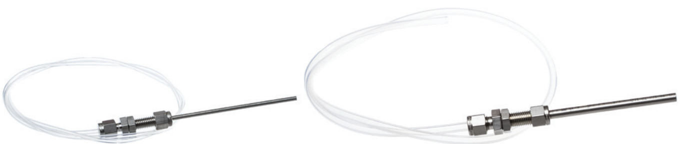

Figure 15. The fraction collection assembly for the Flash 75 system (left) and the Flash 150 system (right).

Note: All the alligator clips must be connected to metal fittings/objects, not to the plastic tubing.

Note: Before installing the grounding kit, ensure that the system tubing has been installed in accordance with the instructions in “Attach the Inert Gas Supply and Solvent Lines” on page 10.

1. Connect the first alligator clip (item 1 in Figure 16) to a grounded point, such as a metal cold water pipe.

   Note: To be an acceptable grounding point, the entire run of pipe must be made of metal. Have a qualified person, such as an electrician, verify that the point is grounded before operating the system.

2. Connect the next alligator clip (item 2 in Figure 16) to the on/off valve on the top of the solvent reservoir, at the point where the metal tubing connector is located.
3. Connect the next alligator clip (item 3 in Figure 16) to the three-way valve on the top of the sample injection module, at the point where the metal tubing connector is located.
4. Connect the next alligator clip (item 4 in Figure 16) to the bottom of the sample injection module, at the point where the metal tubing connector is located.
5. Connect the next alligator clip (item 5 in Figure 16) to the three-way valve on the top of the radial compression module, at the point where the metal tubing connector is located.
6. Connect the last alligator clip (item 6 in Figure 16) to the metal fitting on the bottom of the radial compression module.
7. Connect the fraction collection assembly tube (item 7 in Figure 16) to the fitting on the bottom of the radial compression module as described in “Fitting with Nut and Ferrules” on page 10.

   Note: The grounding kit contains two fraction collection assemblies. The one with the smaller nozzle and a tube with an outer diameter of 1/8" is for Flash 75 and the one with the larger nozzle and a tube with an outer diameter of 1/4" is for Flash 150; see Figure 15.

8. Wrap the uninsulated wire (item 8 in Figure 16) that connects to the last alligator clip (item 6 in Figure 16) around the fraction collection tube (item 7 in Figure 16) and then securely tighten the terminal ring on the uninsulated wire (item 9 in Figure 16) between the two hex nuts on the fraction collection nozzle (item 10 in Figure 16).
9. Verify that the system is grounded using an electrical multimeter (DVM). Nominal resistance must be below 5 Ohm between ground and each metal point in the system.

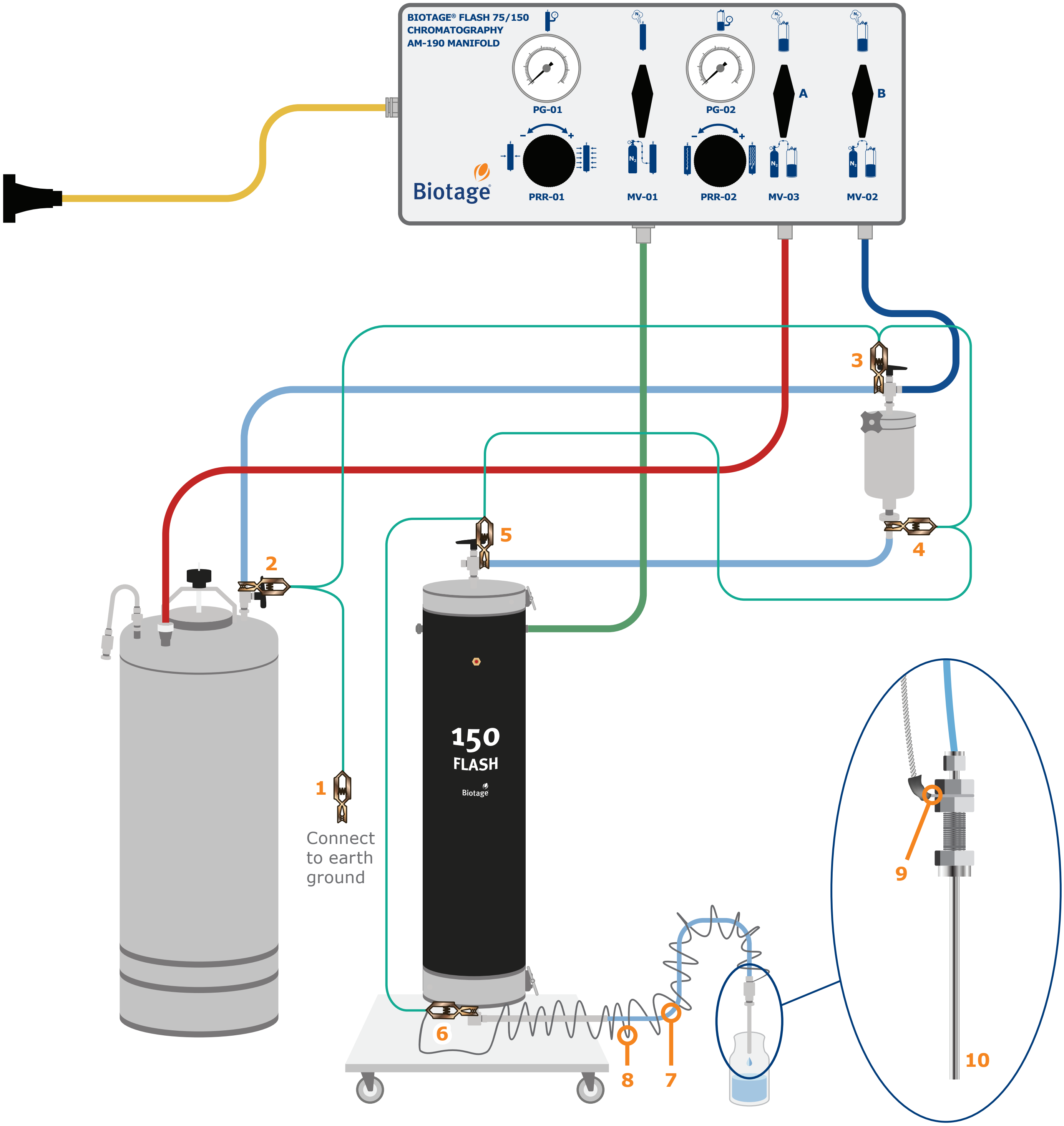

Figure 16. System grounding schematic. Note that all the alligator clips must be connected to metal fittings/objects, not to the plastic tubing.

How to Use the System

Warning

» Before operating the system, please read and observe the safety requirements in the “Safety” section on page 7.

Note: We recommend that you keep a logbook of all the runs performed on the system (with the date, time, user, used solvent or solvent mixture, and pressure settings for each run) to keep track of the pressurized cycles.

Fill the Solvent Reservoir

1. Ensure that the two-way valve on the top of the solvent reservoir is closed; see Figure 17.
2. Ensure that the inert gas supply is turned off and that the three valves on the AM-190 manifold are set to their off/vent positions (see Table 2 on page 3).
3. Take the necessary precautions to avoid exposure to harmful gases and open the lid by loosening the clamp screw and then tilting the lid.
4. Ensure that the system is grounded; see “Ground the System” on page 12.
5. Fill the solvent reservoir with the desired solvent.

   Note: If the solvent reservoir is not empty, ensure that you fill it with the same solvent or solvent mixture.

6. Put the lid back in place by tilting and lowering it into the mouth of the solvent reservoir. Hand-tighten the clamp to secure the lid.

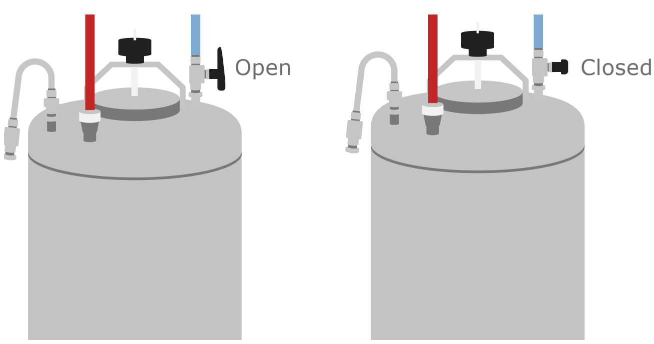

Figure 17. The two-way on/off valve on the solvent reservoir open (left) and closed (right).

Insert a Flash Cartridge

Warning

» Never run the system without a cartridge, or with the wrong size of cartridge.

» Never loosen the V-band clamps when the radial compression module is under pressure. A red pressure indicator on the radial compression module sticks out when the pressure is above 20 psi (1.4 bar); see Figure 2 on page 2.

» Inspect the V-band clamp before and after each use. The clamp must be replaced when it shows any signs of wear and tear, or at 500 pressure cycles, whichever comes first. Only use clamps and screws supplied by Biotage.

» Confirm that the V-band clamp is tight before compressing the radial compression module. Only hand-tighten the clamp to a maximum torque of 100 lbf-in (11.3 Nm). If the cartridge is not sealed properly, replace the O-ring and retry, or contact Biotage 1-Point Support.

1. Ensure that the three-way valve on the radial compression module and the two-way valve on the top of the solvent reservoir are closed.
2. Ensure that the inert gas supply is turned off and that the three valves on the AM-190 manifold are set to their off/vent positions (see Table 2 on page 3).
3. Remove any outer plastic wrapping from the cartridge. You may wish to keep label information for your records.
4. Remove the end caps from the cartridge and keep them.
5. Loosen the upper and lower V-band clamps.
6. Remove the top head assembly from the radial compression module.
7. Lower the cartridge into the radial compression module and confirm that it is level with the top of the barrel.

   Note: If the cartridge does not go all the way into the radial compression module, loosen the lower V-band clamp until the cartridge top is level with the top of the barrel.

8. When the cartridge is level with the top of the barrel, ensure that the O-ring is secure inside the groove in the top lip of the barrel and that it is not damaged; see Figure 26 on page 19.

   Note: If the O-ring shows any signs of wear or damage, replace it. Two spare O-rings are supplied with the system.

9. Place the top head assembly back in place and hand-tighten the V-band clamps alternating between the upper and lower until secure.

   Tightening the V-band clamp will engage the knife-edge seal into the cartridge and engage the O-ring to seal the inert gas pressure within the barrel. Note that the V-band clamp should not be over-tightened. Maximum recommended tightening torque is 100 lbf-in (11.3 Nm).

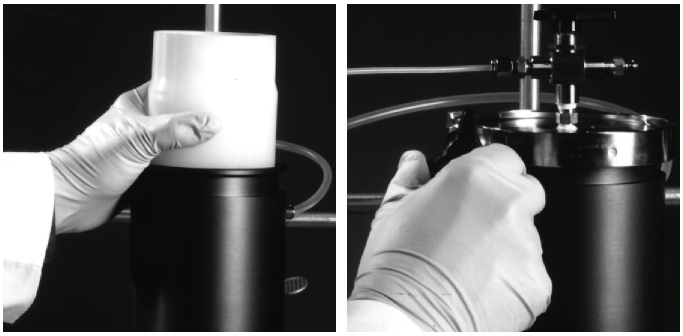

Figure 18. Inserting a flash cartridge into the radial compression module.

Pressurize the Radial Compression Module and the Solvent Reservoir

Warning

- The system is operated using pressurized inert gas. Ensure that all seals are properly mounted and that all clamps are properly closed before introducing pressurized gas into the system.
- To avoid injury to yourself or damage to the system, set the inert gas supply pressure to between 100 and 125 psi (6.9 to 8.6 bar).
- Follow all applicable safety procedures when working with bottled gas.

1. Ensure the system is grounded; see "Ground the System" on page 12.
2. Ensure that the three-way valve on the radial compression module and the two-way valve on the solvent reservoir are closed.
3. Ensure that all the lines are properly connected as described in "Attach the Inert Gas Supply and Solvent Lines" on page 10 and that there is solvent in the solvent reservoir.

   Note: If using a SIM, the solvent tube must be connected between the solvent reservoir and the radial compression module as shown in the left schematic in Figure 13 on page 11 for you to be able to equilibrate the cartridge. The solvent tube may contain solvent; please ensure to take the necessary precautions to avoid spillage when disconnecting the tube.
4. Turn on the inert gas supply that is connected to the AM-190 manifold (see A in Figure 4 on page 3). Confirm that the gas pressure is between 100 and 125 psi (6.9 to 8.6 bar).
5. Pressurize the radial compression module by turning the **MV-01** valve on the AM-190 manifold to the on/pressurize position (see Table 2 on page 3).
6. Set the radial compression pressure to 100 psi (6.9 bar) by adjusting the **PRR-01** regulator on the AM-190 manifold.
7. Confirm that the cartridge is compressed; the red pressure indicator on the front of the radial compression module sticks out (see Figure 2 on page 2).
8. Pressurize the solvent reservoir by turning the **MV-03** valve on the AM-190 manifold to the on/pressurize position (see Table 2 on page 3).
9. Set the equilibration flow rate/solvent pressure by adjusting the **PRR-02** regulator on the AM-190 manifold as follows:
   - Flash 75M: approx. 30 psi (2.1 bar)
   - Flash 75L and Flash 150M: approx. 45 psi (3.1 bar)
   - Flash 150L: approx. 45 to 60 psi (3.1 to 4.1 bar)

   Note: These solvent pressure settings are approximate guidelines. Your particular mobile phase, depending on its viscosity, may require a higher or lower pressure.

   Note: Ensure that the solvent pressure is at least 20 psi (1.4 bar) lower than the radial compression pressure.

Equilibrate the Cartridge

Warning

- Always equilibrate the cartridge.
- Equilibrate the cartridge at a low flow rate to avoid extreme temperature rise.
- Only use waste/fraction collection vessels that are grounded or made of glass.
- If the system is placed in a walk-in fume hood, a fume extractor must be used for open waste/fraction collection vessels.

1. Ensure that the fraction collection nozzle is inserted into a collection vessel that is grounded or made of glass. If the system is placed in a walk-in fume hood, a fume extractor must be used for open collection vessels.
2. Turn the three-way valve on the radial compression module toward the tube connected to the solvent reservoir.
3. Open the two-way valve on the solvent reservoir and allow solvent to flow through the cartridge.
4. When solvent exits from the fraction collection nozzle, close the two-way valve on the solvent reservoir and empty the collection vessel.

Load Sample and Collect Fractions

A liquid sample or a solid sample that has been pre-adsorbed on silica can be loaded onto the equilibrated cartridge using a sample injection module (SIM); see Figure 19 and Figure 20.

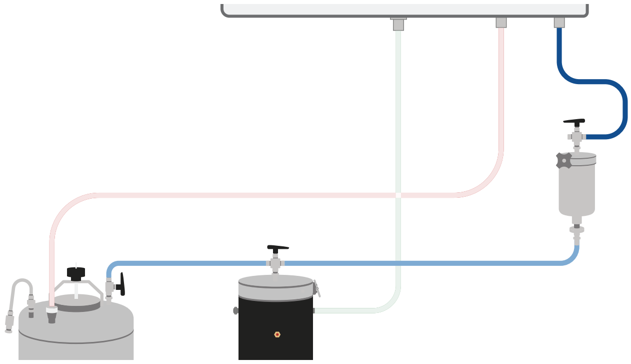

Figure 19. Liquid sample loading using a sample injection module (SIM).

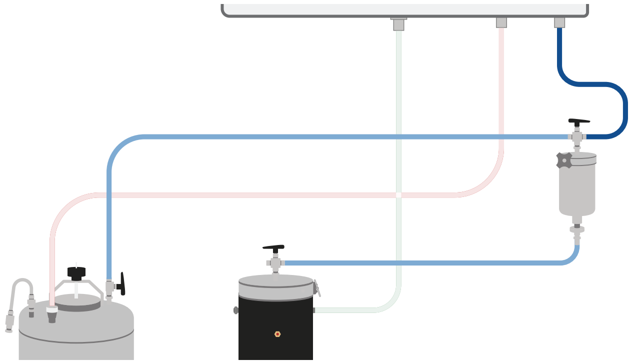

Figure 20. Solid sample loading using a sample injection module (SIM).

Alternatively, if the sample is soluble in a small volume of solvent, it can be injected with a syringe through a Luer-lock injection port on the radial compression module; see Figure 21. The port is fitted by disconnecting the SIM outlet tube from the radial compression module and replacing it with the Luer-lock injection port (supplied with Flash 150 systems).

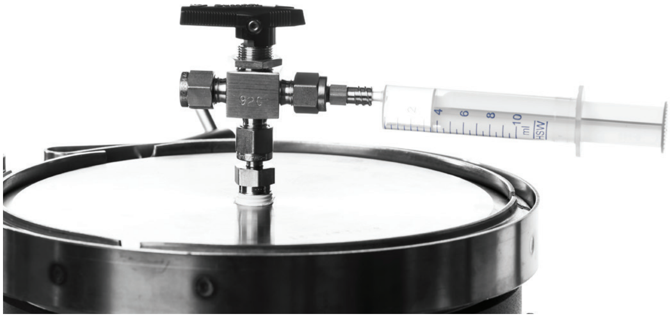

Figure 21. Alternatively, if the sample is soluble in a small volume of solvent, it can be injected with a syringe through a Luer-lock injection port on the radial compression module.

Load the Sample into the SIM

1. Ensure that the **MV-02** valve on the AM-190 manifold is set to its off/vent position (see Table 2 on page 3) and the two-way valve on the solvent reservoir is closed.
2. If loading a solid sample, close the three-way valve on the radial compression module and disconnect the solvent tube connected between the solvent reservoir and the radial compression module from the module and connect it to the SIM; see Figure 20.

   Note: The solvent tube contains solvent; please ensure to take the necessary precautions to avoid spillage when disconnecting the tube.
3. Insert a bottom frit into the SIM's frit holder:
   a. Push one bottom frit into a sealing ring.
   b. Unscrew the lower half of the frit holder and insert the sealing ring and frit into the lower half; see Figure 22.

      Note: Ensure that the plastic frit is at the bottom of the frit holder.
   c. Screw the lower half of the frit holder back in place. Finger-tight is normally adequate.

Figure 22. Inserting a bottom frit into the lower half of the frit holder.

4. Loosen the clamp on the SIM and remove the top assembly (clamp, lid with the three-way valve, and sanitary gasket).
5. Insert a top frit of the correct diameter into the SIM and push it down to the bottom using the frit insertion tool included in the SIM kit; see Figure 23.

Figure 23. Inserting a frit into the SIM 2000 body using its frit insertion tool. Note that the insertion tool for SIM 500 and SIM 1000 does not have a handle.

6. Pour the liquid sample or the silica with the adsorbed sample into the SIM. See Table 4 for maximum volumes.

   Note: When working with oils, dilute the sample with elution solvent until it will flow freely down a glass rod.

SIM	Max Volume (mL)	Max Silica Mass (gram/SIM Unit*)
500	350	175
1000	900	450
2000	1800	900

Table 4. Maximum volume and nominal mass of silica for the SIM modules.

* This is the maximum amount of silica that fits into the SIM; the actual amount of silica used will depend on the sample being loaded.

7. If loading a solid sample:
   a. Tap the silica down using the frit insertion tool.
   b. Insert a second top frit into the SIM and push it down using the frit insertion tool.

      Note: The top frit must be installed or the separation efficiency will be low.
8. Ensure that the gasket grooves of the lid and SIM body are free from any particulates.
9. Place the top assembly back in place and tighten the clamp. Ensure that the sanitary gasket is captured in the grooves of the lid and SIM body; see Figure 24.

   Note: Use a sanitary gasket that compatible with the used solvent; see "Sanitary Gaskets" on page 4.

   Note: If the gasket shows any signs of wear or damage, replace it.

   Note: It is sometimes useful to apply a small amount of solvent to the groove and gasket to ensure a good seal.
10. To load the sample onto the equilibrated cartridge and elute fractions, see "Load the Sample onto the Cartridge and Collect Fractions" below.

Figure 24. The sanitary gasket must be captured in the grooves of the lid and the SIM body.

Load the Sample onto the Cartridge and Collect Fractions

Collect your fractions in flasks that are grounded or made of glass. The following are guidelines for vessel types by cartridge volume:

» Flash 75M (400g): 125-mL flasks
» Flash 75L (800g): 250-mL flasks
» Flash 150M (2.5 kg): 1-liter flasks
» Flash 150L (5.0 kg): 2-liter flasks

Since your compound will not elute in less than one column volume (CV), you may wish to collect the first 0.5, 1, or 3 to 6 liters in a larger flask or carboy. As you approach the point where your compound will elute, use the smaller fraction collection vessels (see the bullet list above) to maximize your yield and purity.

Note: Based on the predicted retention volume (CP) of the first compound, the initial solvent volume could be collected in one large flask to minimize glassware usage.

Warning

» Only use waste/fraction collection vessels that are grounded or made of glass.

11. If the SIM contains a liquid sample:
    a. Ensure that the fraction collection nozzle is inserted into a collection vessel that are grounded or made of glass.
    b. Turn the three-way valve on the radial compression module toward the tube connected to the SIM
    c. Turn the **MV-02** valve on the AM-190 manifold to its on/pressurize position (see Table 2 on page 3) to drive your sample onto the cartridge.
    d. When the sample has been loaded onto the cartridge, i.e. when the SIM outlet tubing is dry, turn the **MV-02** valve on the AM-190 manifold to its off/vent position (see Table 2 on page 3).
12. Turn the three-way valve on the SIM toward the tube connected to the solvent reservoir.
13. Ensure that the solvent reservoir is pressurized, i.e. the **MV-03** valve on the AM-190 manifold is turned to its on/pressurize position (see Table 2 on page 3).
14. Set the solvent flow rate/solvent pressure by adjusting the **PRR-02** regulator on the AM-190 manifold as follows:

    » Flash 75M: approx. 40 psi (2.8 bar)
    » Flash 75L and Flash 150M: approx. 60 psi (4.1 bar)
    » Flash 150L: approx. 60 to 80 psi (4.1 to 5.5 bar)

    Note: These solvent pressure settings are approximate guidelines. Your particular mobile phase, depending on its viscosity, may require a higher or lower pressure. For flash cartridges packed with KP-Sil silica, Biotage recommends a solvent flow rate between 100 and 250 mL/min for Flash 75 and between 400 and 1000 mL/min for Flash 150.

    Note: Ensure that the solvent pressure is at least 20 psi (1.4 bar) lower than the radial compression pressure.

15. Ensure that the fraction collection nozzle is inserted into a collection vessel of a suitable size. See the guidelines above.

    Note: If using a solid sample, the sample has not yet been loaded onto the cartridge. It will be loaded when you open the two way valve on the solvent reservoir in the next step.

16. Open the two-way valve on the solvent reservoir. Solvent will then flow through the SIM and cartridge (solid sample) or cartridge (liquid sample) and out of the fraction collection nozzle.
17. Once your separation is finished, stop the solvent flow by closing the two-way valve on the solvent reservoir.
18. Turn the **MV-03** valve on the AM-190 manifold to its off/vent position (see Table 2 on page 3).
19. Close the three-way valve on the radial compression module.

Refill the Solvent Reservoir

If you need to refill the solvent reservoir during a run:

1. Close the two-way valve on the solvent reservoir.
2. Depressurize the solvent reservoir by turning the **MV-03** valve on the AM-190 manifold to its off/vent position (see Table 2 on page 3).
3. When the pressure has been released, open the lid by loosening the clamp screw and then tilting the lid.
4. Ensure that system is grounded; see “Ground the System” on page 12.
5. Take the necessary precautions to avoid exposure to harmful gases and then fill the solvent reservoir with the desired solvent.

   Note: If changing solvent, the new solvent must be miscible with the previous solvent.

6. Put the lid back in place by tilting and lowering it into the mouth of the solvent reservoir. Hand-tighten the clamp to secure the lid.

Empty the Solvent Reservoir

Warning

» Only use waste/fraction collection vessels that are grounded or made of glass.

If you want to empty the solvent reservoir after the run, this is preferably done before depressurizing the radial compression module.

1. Ensure that the three-way valve on the radial compression module and the two-way valve on the solvent reservoir are closed.
2. Disconnect the solvent tube from the radial compression module and insert it into a collection vessel of a suitable size.
3. Turn the **MV-03** valve on the AM-190 manifold to its on/pressurize position (see Table 2 on page 3).
4. Lower the solvent pressure to 15 psi (1 bar).
5. Take the necessary precautions to avoid exposure to harmful gases.
6. While firmly holding down the solvent tube into the collection vessel, slowly open the two-way valve on the solvent reservoir.

Depressurize the Radial Compression Module and Remove the Used Cartridge

Warning

» Never loosen the V-band clamps when the radial compression module is under pressure. A red pressure indicator on the radial compression module sticks out when the pressure is above 20 psi (1.4 bar); see Figure 2 on page 2.

» Only use waste/fraction collection vessels that are grounded or made of glass.

1. If using a Flash 150 system, reduce the solvent pressure to less than 50 psi (3.5 bar) by adjusting the **PRR-02** regulator before blowing down the radial compression module.
2. Insert the fraction collection nozzle into a collection vessel that are grounded or made of glass.
3. Turn the three-way valve on the radial compression module toward the tube connected to the SIM.
4. Turn the three-way valve on the SIM toward the BLUE tubing that is connected to the AM-190 manifold.
5. Turn the **MV-02** valve on the AM-190 manifold to its on/pressurize position (see Table 2 on page 3)to blow inert gas through the SIM and cartridge and drive out any remaining solvent.
6. When the last of the solvent drains out of the radial compression module, turn the **MV-02** and **MV-01** valves on the AM-190 manifold to their off/vent positions (see Table 2 on page 3).
7. When the radial compression module is <u>not</u> under pressure, i.e. the red pressure indicator on the front of module is <u>not</u> sticking out (see Figure 2 on page 2), turn off the inert gas supply to the system and allow any pressure to dissipate through the system.
8. Loosen the upper V-band clamp and remove the top head assembly from the radial compression module.
9. Remove the cartridge using the cartridge extraction tool supplied with the system; see Figure 25.
10. Put the end caps back on the cartridge and dispose of it in a safe manner.

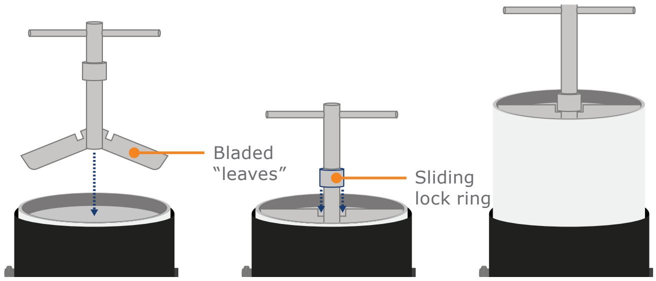

Figure 25. The cartridge extraction tool simplifies the removal of a used cartridge from the radial compression module.

11. Clean the radial compression module using a detergent suitable for the residues and allow it to dry.

    Note: If an O-ring at the top or the bottom of the barrel shows any signs of wear or damage, replace it. Two spare O-rings are supplied with the system.

Figure 26. The O-ring at the top of the radial compression barrel.

12. If the Flash 75/150 system is not used for a longer period of time or you want to change solvent before the next run, please empty the solvent tube that is connected to the solvent reservoir.

    Note: The solvent tube contains solvent; please ensure to take the necessary precautions to avoid spillage when disconnecting the tube.

Clean the Sample Injection Module (Biotage® SIM)

1. Ensure that the three-way valve on the radial compression module and the two-way valve on the solvent reservoir are closed.
2. Ensure that the inert gas supply is turned off and that the three valves on the AM-190 manifold are set to their off/vent positions (see Table 2 on page 3).
3. Disconnect the two grounding clips attached to the SIM (item 3 and 4 in Figure 16 on page 13).
4. Loosen the clamp on the SIM and remove the top assembly (clamp, lid with the three-way valve, and sanitary gasket).
5. Disconnect the SIM outlet tubing from the SIM.
6. Remove the SIM from the chain clamp.
7. If a pre-adsorbed sample was used:
    a. Remove the upper frit in the SIM body; use a screwdriver or similar tool to push through the frit and pull it out.
    b. Scrape out the pre-adsorbent media using a spatula or similar tool and dispose of it in a safe manner.
8. Remove the lower frit in the SIM body; use a screwdriver or similar tool to push through the frit and pull it out.
9. Unscrew the lower half of the frit holder and remove and dispose the sealing ring and frit in a safe manner; see Figure 22 on page 16.
10. Clean the SIM body and the frit holder thoroughly using a detergent suitable for the residues and allow it to dry.
11. When the SIM is dry, reassemble it.
12. Place the SIM back onto the fume hood's mounting bars using the chain clamp.
13. Re-attach the two grounding clips as shown in Figure 16 on page 13.

Troubleshooting

Biotage Flash systems are robust and operate with a minimum amount of required maintenance. This section explains how to troubleshoot some commonly occurring situations where the system, or one of its components, does not seem to be working properly. The most common occurrences are simple gas leaks. To locate the source of a leak, we recommend that you apply a leak detection liquid or soapy water to the suspected area. Once the leak has been located, adjustments or repairs are relatively straightforward. However, if any assistance is needed, we invite you to contact Biotage 1-Point Support; see contact information on the back of this document or visit our website www.biotage.com.

AM-190 Manifold

Problem	Possible Cause	Possible Solution
Gas leaks from the safety relief valve.	1. Gas supply pressure is too high. 2. Safety relief valve is defective. 3. Safety relief valve is not properly installed.	1. Set the inert gas supply pressure to between 100 and 125 psi (6.9 to 8.6 bar). 2. Order a new safety relief valve (P/N 02196). 3. Remove and re-apply PTFE tape, and then re-install using the appropriate wrench.
Inert gas tubing blows/pulls free.	1. Tubing is damaged. 2. Instant-tight fitting is defective.	1. Cut a new end on the tubing, re-insert, and repeat the "pull test". 2. Replace the instant-tight fitting (P/N 02184).
Pressure gauge is stuck.	Pressure gauge is defective.	Replace the pressure gauge (P/N 07900).
Water in the inert gas tubing or the solvent reservoir.	Water trap is full.	Drain the water trap; place a waste container underneath the trap and loosen the screw at the bottom of the trap (see Figure 4 on page 3). Check the inert gas supply for excessive moisture content.
Gas leaks in the AM-190 manifold.	Modular connector screws have loosened.	Contact Biotage 1-Point Support; see contact information on the back of this document or visit our website www.biotage.com.

Biotage® Flash 75/150 Radial Compression Module

Problem	Possible Cause	Possible Solution
Gas leaks from top or bottom of the radial compression module.	1. V-band clamp is loose. 2. O-ring seal is leaking.	1. Tighten the upper V-band clamp. Sometimes it helps to loosen the lower V-band clamp just a bit before tightening the upper, and then re-tighten the lower V-band clamp. 2. Remove the head assembly and replace the O-ring (P/N 00778 for Flash 75 and P/N 01420 for Flash 150).
Gas leaks from the safety relief valve.	1. Radial compression pressure is too high. 2. Safety relief valve is defective. 3. Safety relief valve is not properly installed.	1. Set the radial compression pressure to between 80 and 100 psi (5.5 to 6.9 bar). 2. Replace the safety relief valve (P/N 00790 for Flash 75 and P/N 02196 for Flash 150). 3. Remove and re-apply PTFE tape, and then re-install using the appropriate wrench.
Top head assembly will not seat.	1. Bottom head assembly is out of alignment. 2. The cartridge is too long.	1. Loosen the bottom head assembly, re-align to center, and tighten the V-band clamp. 2. Contact Biotage; see contact information on the back of this document or visit our website www.biotage.com.
Gas bubbles in the collection line.	1. Solvent reservoir is empty. 2. Knife edge seal leaks.	1. Refill the solvent reservoir as described on page 18. 2. Tighten the upper and lower V-band clamps.
Solvent leaks inside of the barrel.	1. Radial compression pressure is too low. 2. Solvent pressure is too high. 3. Knife edge seal leaks.	1. Set the radial compression pressure to between 80 and 100 psi (5.5 to 6.9 bar). 2. Ensure that the solvent pressure is at least 20 psi (1.4 bar) lower than the radial compression pressure. 3. Remove the cartridge and check the top and bottom lip for full penetration. Install a new cartridge if necessary.
Flow runs backward toward the SIM.	Solvent pressure is higher in the cartridge than in the SIM.	Always wait until all flow stops from the fraction collection line before opening the three-way valve on the radial compression module toward the SIM.
Inert gas tubing blows/pulls free.	1. Tubing is damaged. 2. Radial compression pressure is too high. 3. Instant-tight fitting is damaged.	1. Cut a new end on the tubing, re-insert, and repeat the "pull test". 2. Set the radial compression pressure to between 80 and 100 psi (5.5 to 6.9 bar). 3. Replace the instant-tight fitting (P/N 02184).

Operation

Problem	Possible Cause	Possible Solution
No flow.	1. No pressure on the SIM. 2. Flow path incorrect. 3. No pressure on the solvent reservoir. 4. No solvent. 5. Installation incorrect.	1. Open the solvent tank B inert gas supply valve by turning the MV-02 valve to its on/pressurize position (see Table 2 on page 3). 2. Ensure that all the lines are connected correctly (see "Attach the Inert Gas Supply and Solvent Lines" on page 10) and that the three-way valves on the SIM and the radial compression module are opened in the correct direction as described in the "How to Use the system" chapter on page 14. 3. Open the solvent tank A inert gas supply valve by turning the MV-03 valve to its on/pressurize position (see Table 2 on page 3). 4. Refill the solvent reservoir as described on page 18. 5. See "Installation" on page 9.
Flow rate is too low.	1. Insufficient pressure on the solvent reservoir. 2. Tubing is blocked. 3. Cartridge is plugged.	1. Check if the solvent pressure needs to be increased. 2. Close the two-way valve on the solvent reservoir, disconnect the solvent tubing from the radial compression module (or the SIM) and place it in a suitable waste container, and then open the two-way valve and check the solvent flow rate without the radial compression module and the cartridge in-line. If less than 250 mL/min (Flash 75) or 1 L/min (Flash 150), replace the solvent tubing. 3. Check if the solvent pressure needs to be increased. High viscosity solvents require higher inert gas pressure.
Flow rate is too high.	Pressure on the solvent reservoir is too high.	Check if the solvent pressure needs to be decreased. Low viscosity solvents require lower inert gas pressure.

Sample Injection Module (Biotage® SIM)

Solvent Compatibility

Check the solvent compatibility of the sanitary gasket used with the SIM by soaking it for one hour in your mobile phase. Table 3 on page 4 summarizes the typical solvent compatibility. If a gasket swells in use, please let it dry out overnight in a fume hood before reusing. Biotage recommends replacing a gasket if any tears or scratches appear, or if it leaks solvent at typical operating pressure.

Solvent Reservoir

Problem	Possible Cause	Possible Solution
Gas leaks from the safety relief valve.	1. Solvent pressure is too high. 2. Safety relief valve is defective. 3. Safety relief valve is not properly installed.	1. Set the solvent pressure to between 10 and 80 psi (0.7 to 5.5 bar). 2. Order a new safety relief valve (P/N 08865). 3. Remove and re-apply PTFE tape, and then re-install using the appropriate wrench.
Inert gas tubing blows/pulls free.	1. Tubing is damaged. 2. Instant-tight fitting is defective. 3. Solvent pressure is too high.	1. Cut a new end on the tubing, re-insert, and repeat the "pull test". 2. Replace the instant-tight fitting (P/N 02184). 3. Set the solvent pressure to between 10 and 80 psi (0.7 to 5.5 bar).
Gas leaks around the top of the solvent reservoir.	1. Lid is not aligned. 2. O-ring is defective.	1. Close the two-way valve on the solvent reservoir, release the pressure by turning the MV-03 valve on the AM-190 manifold to its off/vent position (see Table 2 on page 3), and then re-seat the lid. 2. Close the two-way valve on the solvent reservoir, release the pressure by turning the MV-03 valve on the AM-190 manifold to its off/vent position (see Table 2 on page 3), remove the lid, and replace the O-ring if necessary.

Optimizing Flash Separations

The following section is a summary of work completed by Biotage application chemists in an effort to reduce the amount of trial and error that is typically associated with optimizing flash separations. The four guidelines to be addressed will focus on the following issues:

1. Predicting column resolution using TLC.
2. Determining the best solvent strength for flash separations.
3. Determining the best solvent selectivity for flash separations.
4. Determining the optimum column size and sample load.

Column Separations are Governed by ΔCV, Not ΔR_f

When transferring a separation from TLC to column chromatography, the use of ΔR_f as the primary factor to predict the degree of resolution is not necessarily valid.

As shown in Figure 27, a good separation on a TLC plate does not always translate into a good column separation. This is due to the fact that R_f values are inversely proportional to the elution time of a component from a chromatography column (see Table 5). Therefore, a better predictor of column behavior is not ΔR_f, but ΔCV[*]. **Generally, TLC conditions that will provide R_f values in the range of 0.15 to 0.40 should be chosen**, which for column chromatography, would result in CVs of 2.5 to 6.7[†]. Components having R_f values above 0.40 will elute too early (i.e. less than 2.5 CVs) whereas those with values much below 0.15 will consume excessive quantities of solvent.

The following sections address the two parameters used to optimize ΔCV; solvent strength and solvent selectivity.

R_f	CV	R_f	CV
0.90	1.10	0.45	2.20
0.85	1.17	0.40	2.50
0.80	1.25	0.35	2.80
0.75	1.33	0.30	3.33
0.70	1.40	0.25	4.00
0.65	1.54	0.20	5.00
0.60	1.65	0.15	6.67
0.55	1.81	0.10	10.0
0.50	2.00		

Table 5. Predicting elution behavior.

[*] The difference in the number of column volumes in which the two components elute.

[†] Due to normal surface area and pore volume variability, CV values may be different than the values predicted.

Vary Solvent Strength to Maintain R_f Values Between 0.15 and 0.40

Using the previous separation as a guide (see Figure 27), the separation in Figure 28 shows that a significant improvement was obtained by operating with a "slower" solvent system. In this case, the separation was optimized by simply changing the relative proportions of the strong and weak solvents.

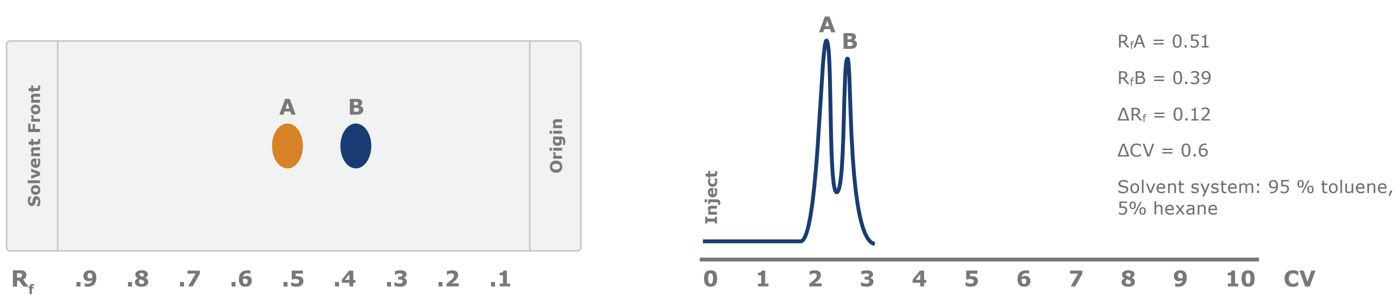

Figure 27. Good separation on a TLC plate does not always translate into a good column separation.

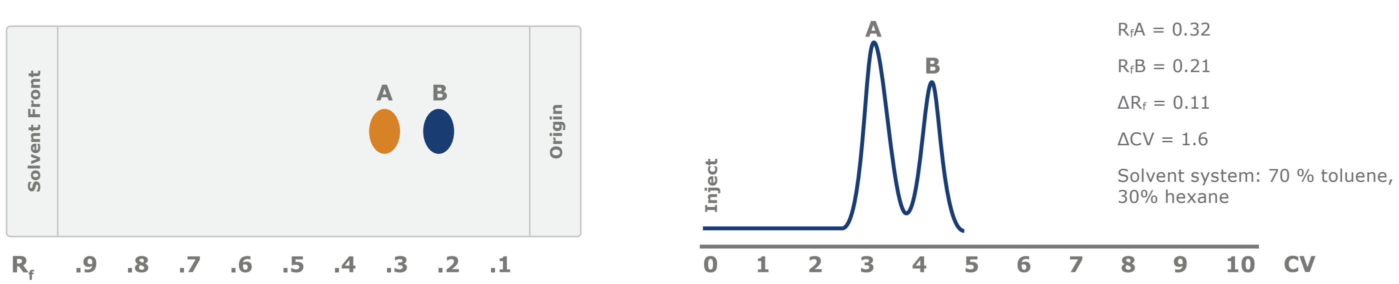

Figure 28. Using a "slower" solvent system (B) can greatly improve the degree of separation.

Vary Solvent Selectivity to Maintain R_f Values Between 0.15 and 0.40

Frequently, the R_f of one component can be changed relative to that of another by varying the "type" of solvent used at a constant solvent strength. The approximate solvent strength of a mixture can be derived by taking the weighted average of the individual solvent strengths. See Table 6 for a listing of commonly used solvents and their strengths.

Solvent Strength Parameters	
Methanol	0.95
Ethanol	0.88
2-Propanol	0.82
Acetonitrile	0.65
Ethyl acetate	0.58
Tetrahydrofuran	0.57
Acetone	0.56
Dichloromethane	0.42
Chloroform	0.40
Diethyl ether	0.38
Toluene	0.29
Hexane	0.01
Heptane	0.01
Iso-Octane	0.01

Example:
1:1 Hexane:Ethyl acetate = (0.5 x 0.01) + (0.5 x 0.58) = 0.295
1:2 Hexane:Dichloromethane = (0.33 x 0.01) + (0.67 x 0.42) = 0.285

Table 6. Solvents commonly used for flash chromatography and their relative strengths.

Solvent Selectivity Groups	
Methanol	II
Ethanol	II
2-Propanol	II
Acetonitrile	VIb
Ethyl acetate	VIa
Tetrahydrofuran	III
Acetone	VIa
Dichloromethane	V
Chloroform	VIII
Diethyl ether	I
Toluene	VII
Hexane	--
Heptane	--
Iso-Octane	--

Table 7. Solvents commonly used for flash chromatography and their relative selectivity group.

In Figure 29, the R_f of the more polar component was changed by replacing ethyl acetate with dichloromethane and resulted in the upper (i.e. less polar) spot being essentially unchanged.

In some instances, varying the polar solvent component can have a much more dramatic effect on the separation, including reversing the elution order of the sample components as shown in Figure 30. Table 7 lists commonly used solvent and their selectivity groups.

For a more detailed discussion of solvent selectivity groups, see *Introduction to Modern Liquid Chromatography* by L. R. Snyder and J. J. Kirkland.

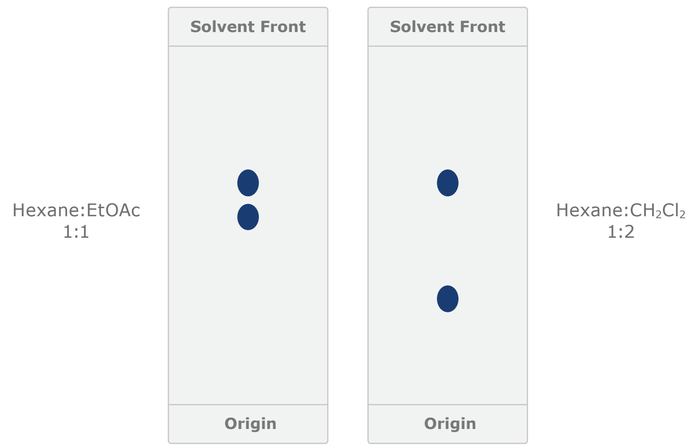

Figure 29. Different solvent mixtures with similar strengths (0.28 and 0.29) will frequently provide different selectivities.

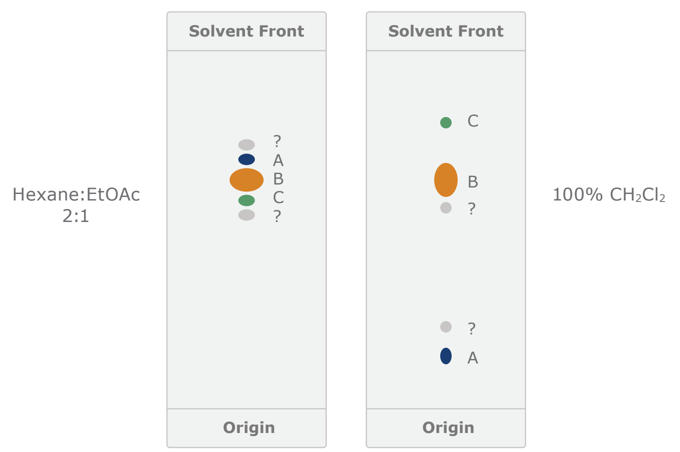

Figure 30. By changing the solvent type (i.e. Group VIa versus Group V), the resolution between B, the compound of interest, and the impurities, changed dramatically. Note that while the R_f values of B are similar on the two plates (0.65 and 0.53) in the example above, the elution order of A and C were reversed.

The Relationship Between the Size of a Column and the Sample Load is Dependent on ΔCV

Table 8 shows recommended sample loads for different length Flash 75 and 150 prepacked cartridges. In general, the more difficult the separation (i.e. a low ΔCV), the less material can be loaded onto the cartridge for a successful/high resolution separation.

Column	Load ΔCV = 0.1–1.9	Load ΔCV = 2.0–3.9	Load ΔCV = 4+
Flash 75M	<4 g	4–20 g	10-40 g
Flash 75L	<8 g	8–40 g	40–80 g
Flash 150M	<25 g	25–125 g	125-250 g
Flash 150L	<50 g	50–250 g	250–500 g

Table 8. Predicting sample loads for prepacked flash cartridges.

Flash Chromatography Workbook

The Biotage Flash Chromatography Workbook has been designed to document the separation steps taken when using a Biotage Flash system to purify synthetic compounds. Upon the completion of a separation using one of the prepacked cartridges from Biotage, you'll be able to evaluate the performance of the Biotage approach versus flash separations using traditional glass columns.

The workbook includes sections on:

- Purification Goals
- Sample/Compound Description
- Current Method
- TLC Method Optimization
- Prepacked Cartridge Data
- Run Analysis
- Fraction Collection
- Results
- Comparison Analysis
- Summary/Conclusions

Flash Chromatography Workbook

Chemist: ______________________

Account: ______________________

Department: ______________________

Address: ______________________

City: __________ State: __________ Zip: __________

Biotage Rep: ______________________ Date: __________

Purification Goals

____

____

____

____

Sample/Compound Description

____

____

____

____

Current Method

Column Size: __________ mm ID x __________ mm long

Silica: __________ grams Vendor: __________

Prep Time: __________ minutes Run Time: __________ minutes

Solvent Used: __________ liters __________

TLC Method Optimization

Solvents Used: ____

Concentration: ____

____ : ____	____ : ____	____ : ____	____ : ____
Solvent Front	Solvent Front	Solvent Front	Solvent Front
Origin	Origin	Origin	Origin

Elution Calculations:

	R_f	CV
A		
B		
C		
D		

	R_f	CV
A		
B		
C		
D		

	R_f	CV
A		
B		
C		
D		

	R_f	CV
A		
B		
C		
D		

$$R_f = \frac{\text{Distance from the origin to the center of the spot}}{\text{Distance from the origin to the solvent front}}$$

$$CV = 1/R_f$$

Prepacked Cartridge Data

Column Size: ____________ mm ID x ____________ mm long ____________ grams of silica

Sample Load: ____________ grams in ____________ mL of ____________

Solvents	Composition	Volume (liters)
Equilibrate:	____________	____________
Wash:	____________	____________
Elute:	____________	____________
	____________	____________
	____________	____________
Strip:	____________	____________

Flow Rate: ____________ mL/min

Solvent Pressure: ____________

Radial Compression Pressure: ____________

Run Analysis

Run Number: ____________ Description: ____________

Fraction Collection

Fraction	Volume (liters)	Comments
1	____________	____________
2	____________	____________
3	____________	____________
4	____________	____________
5	____________	____________
6	____________	____________
7	____________	____________

Fraction Collection (continued)

8 ____________________ ____________________

9 ____________________ ____________________

10 ____________________ ____________________

11 ____________________ ____________________

12 ____________________ ____________________

13 ____________________ ____________________

14 ____________________ ____________________

15 ____________________ ____________________

16 ____________________ ____________________

17 ____________________ ____________________

18 ____________________ ____________________

19 ____________________ ____________________

20 ____________________ ____________________

Results

Fraction #

Solvent Front	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Origin	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Pools: ____________________

Recovered Wt.: ____________________

Comparison Analysis

	Flash Cartridge		Glass Column	
Sample Size:	________________	grams	________________	grams
Flow Rate:	________________	mL/min	________________	mL/min
Preparation Time:	________________	minutes	________________	minutes
Purification Time:	________________	minutes	________________	minutes
Solvent Used:	________________	liters	________________	liters
Product Yield at Target Purity:	________________		________________	

Summary/Conclusions

General Information

Price List and Ordering Information

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Notes

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Biotage is a worldwide supplier of instruments and accessories designed to facilitate the work of laboratory and process chemists. With our deep knowledge of the industry, academic contacts and in-house R&D teams, we can deliver the best solutions to your challenges. We take great pride in our flexibility and ability to meet our customer's individual needs. With strong foundations in both analytical, organic and process chemistry, we can offer the widest range of solutions available on the market.

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