



## FPLC System Purchasing Guide: GMI Certified Knowledge

Frequently used in biochemistry, biology, and enzymology, Fast Protein Liquid Chromatography (FPLC) is a type of liquid chromatography technique that separates proteins, peptides, nucleic acids and other biomolecules from complex mixtures in a liquid phase. Commonly performed as a part of a bigger lab workflow, FPLC is a form of medium-pressure chromatography, offering advantages such as identification of protein profiles present in body fluids like plasma, urine, and cerebrospinal fluids as well as purification of plasmid DNA, RNA, and synthetic nucleotides to name a few.

A basic FPLC system is typically composed of a pump, injection loop and valve, column, UV detector, conductivity meter, as well as a fraction collector and generally utilizes higher flow rate and lower pressures to pass buffers and samples through a separating column. Good FPLC systems offer several advantages like flexibility to support protein purification techniques for affinity, ion-exchange, hydrophobic-interaction, gel-filtration, reverse-flow, multimodal and size-exclusion chromatography and the ability to accommodate high resolution chromatography media of 5-15  $\mu\text{m}$  beads automation for unattended operation

Used for many applications spanning a wide range of wavelengths, it is vital for scientific laboratories and academic foundations to acquire an FPLC system that can deliver reliable and reproducible and can minimize the risk of errors. With a plethora of models and types of FPLC equipment in the market, finding the most appropriate one for your application can be quite a daunting and overwhelming task. Don't fret as GMI has prepared this simple guide to walk you through factors you should consider in selecting the right FPLC system for your workroom.

### Review Application Demands and Needs for your FPLC System

A crucial step in selecting the most suitable FPLC system for your laboratory is reviewing what your application requires of you. You can start by answering these questions:

- Are you doing small-scale protein purification or large preparative and scale-up method development? Answering this can help you decide on the size of equipment to get as well as on the features and functionalities that you should be looking out for. For development, scale-up and production processes, you can look into a complete and customizable FPLC system built with all fluid handling components for smooth delivery and mixing such as the GE Amersham [AKTA Pilot](#), [AKTA Explorer](#) and the Knauer [AZURA Pilot Bio LC 100 LPG](#). For smaller purification quantities, you can check out GE's [AKTA Purifier 10](#). For general purification tasks, you can get the [AKTA Prime Plus](#). All of these units are available at GMI.



- How many and what wavelengths would you want to have access to? Check if your application requires maximum flexibility for simultaneous monitoring of multiple wavelengths. If your application calls for an FPLC system with a UV/Vis detector featuring variable wavelength capabilities, you can get the [Knauer AZURA Pilot Bio LC 100](#) or the [AZURA Pilot Bio LC 250](#).
- How many columns would you want hooked up at once? See if you would need multiple column configurations for faster sample analysis. Column options available at GMI include the [Column XK 26/20 with AK 26 Adapter](#), [Column C 10/10](#), a [small column holder](#), and a [short column holder for columns 26 mm](#), all compatible with the GE Amersham Pharmacia [AKTA](#) FPLC Series.
- Do you require software and computer controlled components? FPLC systems with pre-programmed application protocols and method templates can minimize sample preparation times. For systems optimized for purification of milligram to gram samples and with pre-designed methods included in its software, you can checkout the Knauer [AZURA Compact Bio LC 50](#), [AZURA Pilot Bio LC 100](#), and the [AZURA Pilot Bio LC 250 LPG](#), all offered by GMI.

Depending on your specific application's requirements, you can also shop for the following [FPLC parts](#) and [FPLC accessories](#):

- Autosampler - aids in quick sample loading on a chromatograph, when getting an autosampler be sure to consider its sample volume range, cycle and load times, precision and accuracy as well as container types
- Fraction collector or valve - used to collect and store in different containers all chromatographically separated materials
- Injection loop and valve - for transferring pre-loaded samples onto columns

Aside from these, ensure you obtain maximum control and optimal sample processing by matching the FPLC system you are planning to buy with the computer and software you are using.

### **Consider Lab Area Requirements where the FPLC will be Situated**

Before you start browsing FPLC systems, review your available lab space first as this can influence the convenience of location and accessibility of your equipment. For a large and open workroom, you can check out modular FPLC systems that can be configured and expanded for access to more detection methods and higher throughputs like the GE [AKTA Pure](#), [AKTA Avant](#) as well as the Knauer [AZURA Bio LC 500](#) and [AZURA Bio LC 1000](#). Consisting of separate



modules that can be stacked, switched, connect to work as one unit, and configured to your requirements, these modular systems bring in exceptional flexibility, allowing you to progressively support the latest technology without purchasing a whole new system every so often. If you have a modest space, you can buy compact and easily upgradeable units instead such as the GE [AKTA Start](#), BioRad [NGC Series](#) or a one-step purification chromatograph like the GE [AKTA Prime](#).

### **Establish and Decide on Budget Allocations for your FPLC Purchase**

Look into your laboratory funding and see how much you are willing to allocate for your FPLC system purchase. If you have sufficient resources on hand, you can always opt for [new FPLC systems](#) for your academic and industrial research needs. Consider instrument lifestyle costs as this can also help you decide whether to buy new units for your lab. If you have a limited budget, you can go for one of the popular options these days, purchasing a used, recertified, and [refurbished-to-specification FPLC system](#). As most Original Equipment Manufacturers (OEMs) have tight schedules in supplying new lab instruments, you might want to go for economically priced units from trusted used equipment suppliers. For any urgent unit replacement needs, backups, or to meet your market demands, you can always rely on industry-leading distributors like GMI for quality and easily available certified pre-owned lab equipment, saving up to 70% of your budget.

### **FPLC Pump Type Preference**

In charge of moving the mobile phase through the column and FPLC system at a specific flow rate and under a constant pressure, pumps ensure the constant flow of the liquid catalyst, essential in avoiding changes in elution time of sample components and result errors.

Most FPLC systems make use of two cylinder-piston pumps, utilizing one for each buffer and mixing the output of both in a chamber. There are also simpler units that operate with a single peristaltic pump designed to draw both buffers from different reservoirs with the help of a proportioning valve and a mixing chamber. Both types allow the fraction of each buffer entering the column to be constantly varied.

Normally made of resistant materials, these pumps offer two types of operation, delivering constant (isocratic pump) and variable (gradient pump) mobile phase composition. Pumps may also vary in terms of flow range, going from milliliters per minute in benchtop systems to liters per minute for industrial scale purifications.



In terms of flexibility, you can consider the following pump types and identify the best one for your application:

- Binary Pumps - can pool and combine two eluents together at the same time, made of two independent pumps that allow flow for each solvent, high-pressure proportioning
- Quaternary Pumps - can pool and combine four different eluents at the same time, made of one pump, low-pressure proportioning

It is vital to get corrosion-resistant FPLC pumps that are compatible not just with your solvents but also with your buffers and solutes. Acquire a unit that can offer constant flow delivery, independent of back pressure and low dead volume to avoid possible issues when it comes to solvent changeover.

### **FPLC Column Chemistries and Types**

FPLC columns are glass or plastic cylinders packed with beads of resin, also known as the stationary phase, and filled with the buffer solution. These tubes are responsible for carrying out separation of sample components and compared to HPLC ones, FPLC columns have a maximum pressure of roughly 3-4 MPa.

Considered as the heart of a chromatograph and can make or break your analysis, getting the best choice of column for your system is critical. Understanding your compounds of interest, such as the physicochemical characteristics of the molecules to be separated and the interactions between these molecules through chromatography media or resins, can help you select the most fit column component of your FPLC system. Choose the right one for your application by familiarizing yourself with different types of columns based on their composition and methods of separation. You should consider column length and particle size/porosity as these factors can affect the efficiency, sensitivity, and speed of your sample analysis. Some FPLC columns are described below for your reference:

- Normal Phase Columns - separate sample components based on polarity of sample components, widely used in pharmaceutical analysis
- Reversed Phase Columns - separate sample components also based on polarity but happens opposite the normal phase columns, based on hydrophobicity
- Ion Exchange Columns - separate sample components based on the attractive ionic force between molecules and charged stationary phase, surface charges
- Size Exclusion/Gel Filtration Columns - separate sample components based on size

In need of columns and column parts compatible with your FPLC system? Browse GMI's convenient online store showcasing a large inventory of liquid chromatography [parts and accessories](#) from well-known scientific brands [here](#).



## FPLC Flow Rate

Besides column dimension, stationary phase particle size, and temperatures, an FPLC system pressure is also affected by the flow rate implemented. Utilizing the most appropriate flow rate is crucial as using a higher than usual one can impact your system's chromatographic quality, offering insufficient time for your analyte to interact with the stationary phase.

Review your column particle size, length, and internal diameter as these will help you identify the right flow you need. Large diameter columns usually require higher flow rates. Shorter columns and elevated flow rates result to faster analyses. You can refer to the table below for the typical flow rates used based on your column's internal diameter, injection volume, and loading capacity.

Internal Diameter (mm)	Typical Injection Volume ( $\mu\text{L}$ )	Loading Capacity (mg)	Typical Flow Rates
4.6	15	1	0.5-2
10	100	4.7	4-15
21.2	400	19.5	10-50
30	1000	42.5	40-100
50	2000	118	100-300
100	10000	473	400-1000

## FPLC Detector Types Required

FPLC detectors are units used to measure the amount of individual molecules eluted off a chromatography column. Picking out the right detector will depend on the analytes you want to detect, the level of required sensitivity, properties of eluents or liquid solvents used in the separation, and the type of information required by your experiment.



Some popular non-destructive FPLC detectors in the market include:

- pH Detector - monitors and measures pH values
- Conductivity Detector - detects ions in a solution, highly sensitive and susceptible to effects of temperature variations, measures inorganic ions and small organic substances such as organic acids and amines
- UV/Vis Single Wavelength Detector - measures a sample's absorption of light on a single wavelength
- UV/Vis Multiple Wavelength Detector - measures a sample's absorption of light on multiple wavelengths and are more sensitive, for routine analysis of a wide range of applications
- UV/Vis Diode Array Detector - presents multiple photodiode arrays to obtain information through a wide range of wavelengths at one time
- Refractive Index Detector - based on changes in refractive index of eluting compounds in the mobile phase, less sensitive compared to a UV-Vis detector
- Fluorescence Detector - provides higher sensitivity compared to a UV-Vis detector

Keep in mind that a good detector offers low noise, low detection limits, low dead volume to eliminate memory effects, high sensitivity towards solute over mobile phase and a large dynamic linear range.

All FPLC components mentioned above are usually offered by manufacturers as part of a complete system. However, at GMI, you are given more flexibility to combine a range of options available for each component depending on your chosen brand such as GE and Knauer. Additionally, GMI also showcases a wide range of [new](#), [used](#), and always in-stock FPLC systems for lab scale chromatographic separations as well as for qualitative and quantitative analyses of your biological compounds.

For over 20 years, GMI has been serving the scientific market and cost conscious laboratories with leading-edge products and unmatched services. With an ISO 9001:2008 certification under our wings, we assure any FPLC System purchased from us has undergone meticulous refurbishing, recalibration, recertification, and testing processes. We also offer a number of service agreement, warranty, rental, installation and training options for our FPLC equipment.

For any assistance needed on potential new or used FPLC system purchase, feel free to reach us at **1-888-702-1775** or email us at [sales@gmi-inc.com](mailto:sales@gmi-inc.com).