Converting the NGC Chromatography System to High Flow

Quick Guide

The Bio-Rad™ NGC Chromatography Systems with F100 System Pumps can precisely generate flow rates from 0.01 to 100 ml/min in standard operation. Flow rates up to 200 ml/min can be reached using the NGC F100 Pump Module with the NGC Buffer Blending Valve Module and the NGC High-Flow Tubing Kit. Here we describe the plumbing configurations and considerations for NGC System operation at high flow rates (>40 ml/min).

Equipment

NGC Chromatography Systems with F100 System Pumps can achieve flow rates up to 100 ml/min. These NGC Chromatography Systems include the Quest 100 Plus (catalog #7880004), Scout 100 Plus (#7880008), Discover 100 (#7880010), and Discover 100 Pro (#7880012). An NGC Column Switching Valve Module (#7884026) is recommended for all high-flow operation, since it adds additional pressure sensing capabilities that will safeguard your columns. We recommend using positions 2, 3, or 4 on the Column Switching Valve for ease of attaching large columns. To increase the flow rate of an NGC Chromatography System to >100 ml/min, an NGC Buffer Blending Valve Module (#7884010) in high-flow mode can double the flow rate of the system to a maximum of 200 ml/min. Ensure that the lengths of tubing connecting the Buffer Blending Valve outlet ports to the F100 System Pumps (#7884003) are equivalent in length. The NGC Chromatography System must be powered off when swapping valve modules, flow cells, and mixer modules.

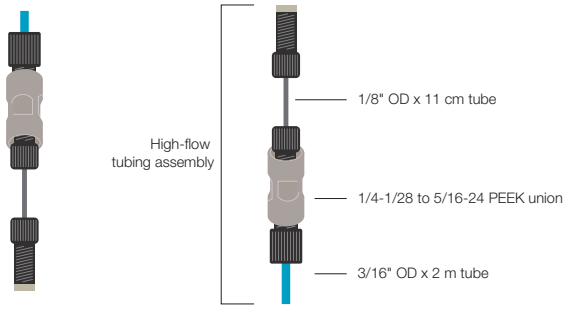
Note: Remove the 20 psi Backpressure Regulator from the NGC System while using any flow rate >20 ml/min.

Component Requirements

Tubing

As the flow rates are increased on the NGC System there is a corresponding increase in the overall system pressure, which can be mitigated by the following tubing modifications for optimal performance. The NGC High-Flow Tubing Kit (#7885171) contains preassembled 3/16" OD FEP Tubing with 5/16-24 fittings, short pieces of 1/8" OD FEP Tubing preassembled with 1/4-28 fittings, and F 1/4-28 to F 5/16-24 PEEK unions to connect them as shown. The 3/16" OD FEP Tubing should be used for buffer inlet lines as well as a final waste line exiting from the fraction collector. The 3/16" OD FEP Tubing is recommended for flow rates from 40 to 100 ml/min. However, it is necessary for flow rates >100 ml/min to prevent pump starvation and cavitation. Prime the pumps and then run the system pump calibration at an elevated flow rate (>80 ml/min) to check flow rate accuracy.

For operating at flow rates >100 ml/min, changing the system tubing past the F100 System Pump from the standard green PEEK (1/16" OD x 0.030" ID) to clear FEP (1/8" OD x 0.0625" ID) for all module-to-module connections is recommended to decrease overall system pressure. This tubing will need to be handmade for your NGC Chromatography System: cut tubing to appropriate lengths, add end fittings, and plumb the NGC System. Instructions for making fittings on tubing are found in your NGC Fittings Kit. Note that wider inner diameter (ID) tubing can also be used at lower flow rates to reduce pressure but will increase the overall swept volume of the system and may decrease peak resolution.



Assembly of the 3/16" OD FEP Tubing with 5/16-24 fittings with the PEEK union for high-flow operation on the NGC Chromatography System. FEP, fluorinated ethylene propylene; OD, outer diameter; PEEK, polyetheretherketone.

Mixer Barrel

The mixer barrel, located on the NGC Mixer Module (#7884018), is available in different sizes for optimal mixing at specific flow rates. The installed mixer barrel should be upgraded to the 5 ml mixer barrel for flow rates up to 40 ml/min and to the 12 ml mixer barrel for flow rates from 40 to 200 ml/min. Instructions for swapping mixer barrels are found in the NGC Chromatography Systems and ChromLab Software Instrument Guide (10000049091).

UV Flow Cell

The 5 mm standard NGC UV Flow Cell (#7885024) on the NGC Single-Wavelength or Multi-Wavelength Detector Module (#7884008 or 7884009, respectively) may need to be changed to a 2 mm preparative NGC UV Flow Cell (#7885022). The 5 mm standard NGC UV/Vis and Conductivity Flow Cell (#12012532) on the NGC Multi-Wavelength II Detector Module (#12010343) should be changed to a 2 mm preparative NGC UV/Vis and Conductivity Flow Cell (#12012533) for all applications using a flow rate ≥80 ml/min. Decreasing the pathlength of the flow cell will decrease the overall sensitivity. This may be needed to prevent detector saturation during large-scale purifications.

Fraction Collector

The BioFrac Fraction Collector (#7410002) can operate at a maximum of 100 ml/min. The NGC Fraction Collector with Racks (#17002070) can operate at up to 200 ml/min. The waste line exiting the BioFrac or NGC Fraction Collector from the divert valve should be updated to the 3/16" OD x 0.125" ID tubing from the NGC High-Flow Tubing Kit when being used at flow rates >40 ml/min.

The NGC Fraction Collector should be plumbed with the 1/8" OD FEP Tubing with a short PEEK Nut and yellow ferrule fitting for both the inlet line entering and waste line exiting the NGC Fraction Collector divert valve. This piece of tubing comes only with the NGC Fraction Collector in the High-Flow Tubing Kit. Standard PEEK Nuts from the NGC Fittings Kit are not compatible on the NGC Fraction Collector. Use one of the 3/16" OD FEP Tubing pieces from the High-Flow Tubing Kit for the final waste line from the magnetic NGC Fraction Collector union to the waste bottle.

Materials

Product	Details	Catalog Number	Quantity
NGC Chromatography System	NGC Quest 100 Plus	7880004	1
NGC Column Switching Valve Module, 100 ml	Column Switching Valve adds additional pressure monitoring capabilities to the NGC System	7884026	1
NGC Buffer Blending Valve Module	For mixing buffer conditions inline on the NGC System or for high-flow operation	7884010	1
BioFrac Fraction Collector	Fractionation up to 100 ml/min	7410002	1
NGC Fraction Collector with Racks	Fractionation up to 200 ml/min	17002070	1
NGC High-Flow Tubing Kit	High-Flow Tubing Kit includes: ■ 5 x 3/16" OD FEP Tubing with 5/16-24 fittings ■ 5 x F 1/4-28 to F 5/16-24 PEEK union ■ 5 x short 1/8" OD FEP Tubing with 1/4-28 fittings ■ 5 x bottle cap with holes for 3/16" OD and 1/8" OD FEP Tubing ■ 1 x NGC Fraction Collector 1/8" OD FEP waste line with short PEEK Nuts ■ 1 x NGC Fraction Collector 1/16" OD green PEEK waste line with short PEEK Nuts Provided with new purchases of 7880004, 7880008, 7880010, and 7880012	7885171	1
1/8" OD FEP Tubing (15')	Use to replace green PEEK system tubing for flow rates >100 ml/min	7500603	1
PEEK Nut 1/8" (package of 10)	Use with 1/8" OD FEP Tubing	7885015	3
PEEK Ferrule 1/8" (package of 15)	Use with 1/8" OD FEP Tubing	7885055	2
NGC UV Flow Cell, 2 mm, preparative	For NGC Single-Wavelength Detector Module (#7884008, discontinued) or NGC Multi-Wavelength Detector Module (#7884009, discontinued)	7885022	1
NGC UV/Vis and Conductivity Flow Cell, 2 mm, preparative	For NGC Multi-Wavelength II Detector Module (#12010343)	12012533	1
NGC F100 Mixer Barrel, 5 ml	For flow rates up to 40 ml/min	7884023	1
NGC F100 Mixer Barrel, 12 ml	For flow rates from 40 to 200 ml/min	7884024	1

FEP, fluorinated ethylene propylene; OD, outer diameter; PEEK, polyetheretherketone; UV/Vis, ultraviolet and visible light.

Precolumn Pressure Adjustment

When running at elevated flow rates (>100 ml/min), the system pressure will be greater than the precolumn pressure, due to ID constriction when flowing through the injection valve. To successfully achieve maximum flow rates on the NGC System, the precolumn pressure limit in the method settings must be adjusted prior to running a column. This is done by measuring the pressure at the desired operating flow rate with no column inline. Calculate the differential pressure between the system pressure and the precolumn pressure — this is the additional pressure imposed by the injection valve at higher flow rates. This calculated difference should be added to the precolumn pressure limit of the column in the method settings and manual settings of the NGC System. Refer to the tables in the Pressure Ranges at High Flow Rates section to compare NGC System pressure differences at multiple flow rates with green PEEK system tubing versus clear FEP system tubing.

Case Study

Upon running the NGC System at 175 ml/min, the difference between the system pressure and the precolumn pressure is found to be 54 psi (system pressure = 134 psi, precolumn pressure = 80 psi). The pressure readings were monitored via the pressure traces in the chromatogram, but these values can also be found either on the module faceplates or in the system control tab in the module view. The column in question for high-flow use has a designated precolumn pressure limit of 74 psi and delta column pressure limit of 44 based on specifications published for the column. To adapt this column for high-flow use, update the precolumn pressure limit to 128 psi (74 + 54 psi) in the method settings in ChromLab Software. This will allow the system to run at the desired 175 ml/min flow rate while considering the pressure generated by the injection valve. The delta column pressure limit should remain at 44 psi to guard the column against true overpressure.

Pressure Ranges at High Flow Rates

Clear FEP Tubing/Beige PEEK Tubing (ID 0.0625")

Flow Rate, ml/min	System Pressure		Precolumn Pressure		Delta Column Pressure	
	psi	MPa	psi	MPa	psi	MPa
100	50–57	0.34–0.39	24–36	0.17–0.25	15–21	0.10–0.14
125	72–82	0.50–0.57	38–52	0.26–0.36	20–31	0.14–0.21
150	96–111	0.66–0.77	54–71	0.37–0.49	28–42	0.19–0.29
175	124–144	0.85–0.99	70–92	0.48–0.63	35–54	0.24–0.37
200	146–167	1.00–1.15	83–107	0.57–0.74	40–63	0.28–0.43

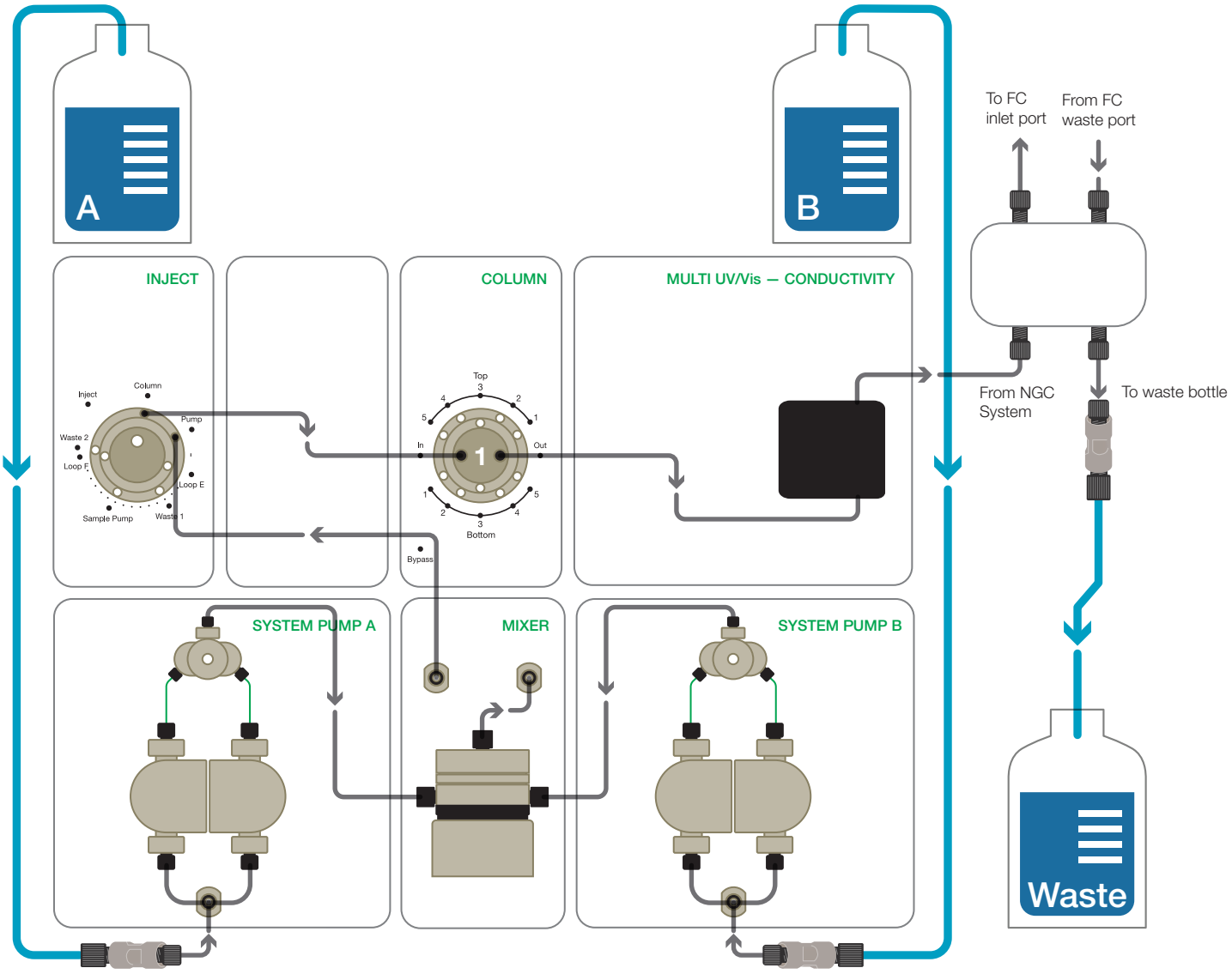
Green PEEK Tubing (ID 0.030")

Flow Rate, ml/min	System Pressure		Precolumn Pressure		Delta Column Pressure	
	psi	MPa	psi	MPa	psi	MPa
20	26–34	0.18–0.23	8–11	0.05–0.07	4–9	0.03–0.06
40	68–77	0.47–0.53	44–54	0.30–0.37	20–31	0.14–0.21
60	124–137	0.85–0.94	82–96	0.57–0.66	39–45	0.27–0.31
80	178–195	1.22–1.34	125–146	0.86–1.01	41–62	0.28–0.43
100	260–282	1.79–1.94	190–218	1.31–1.50	60–90	0.41–0.62

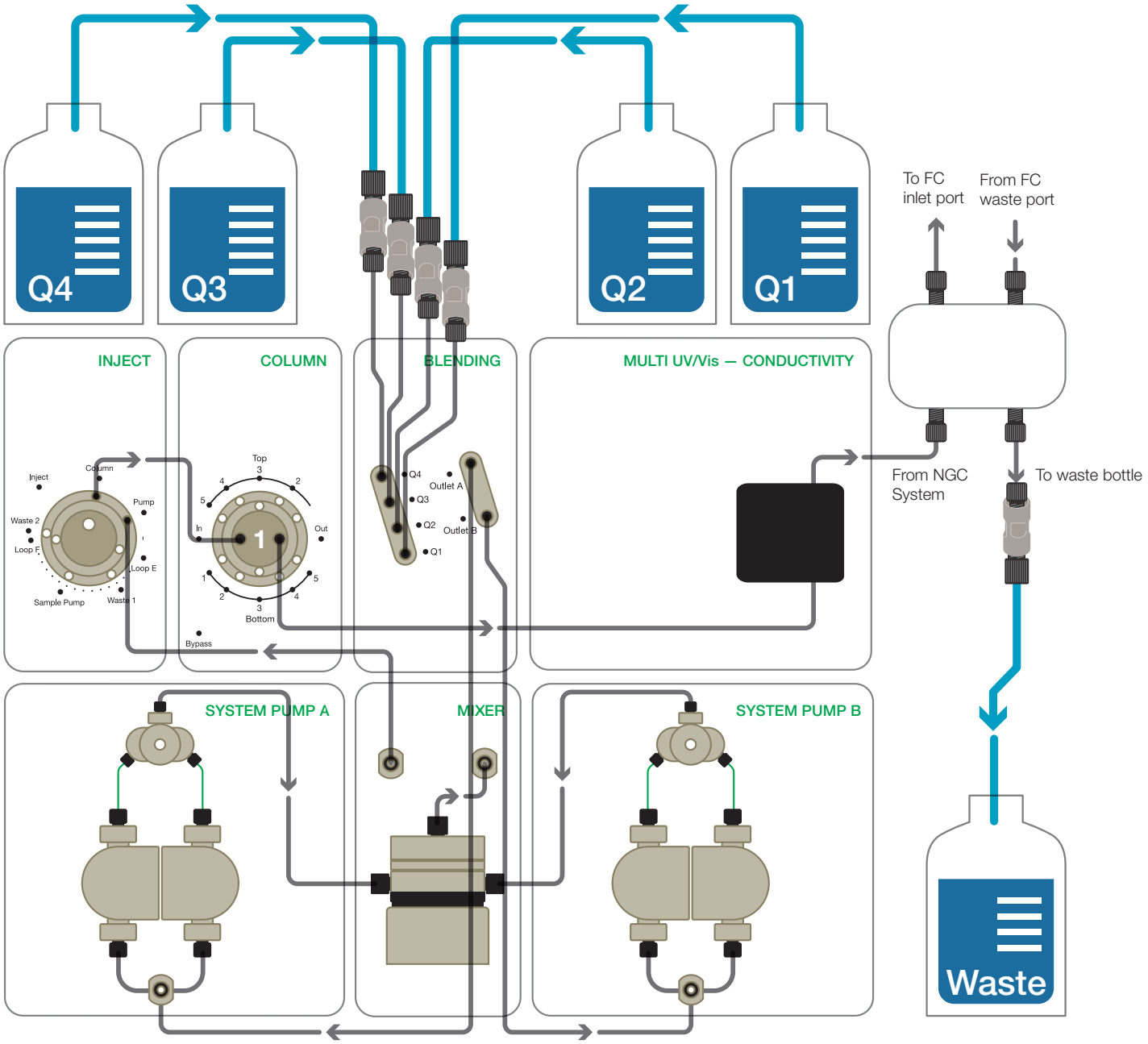
FEP, fluorinated ethylene propylene; ID, inner diameter; PEEK, polyetheretherketone.

Examples of High-Flow Configurations

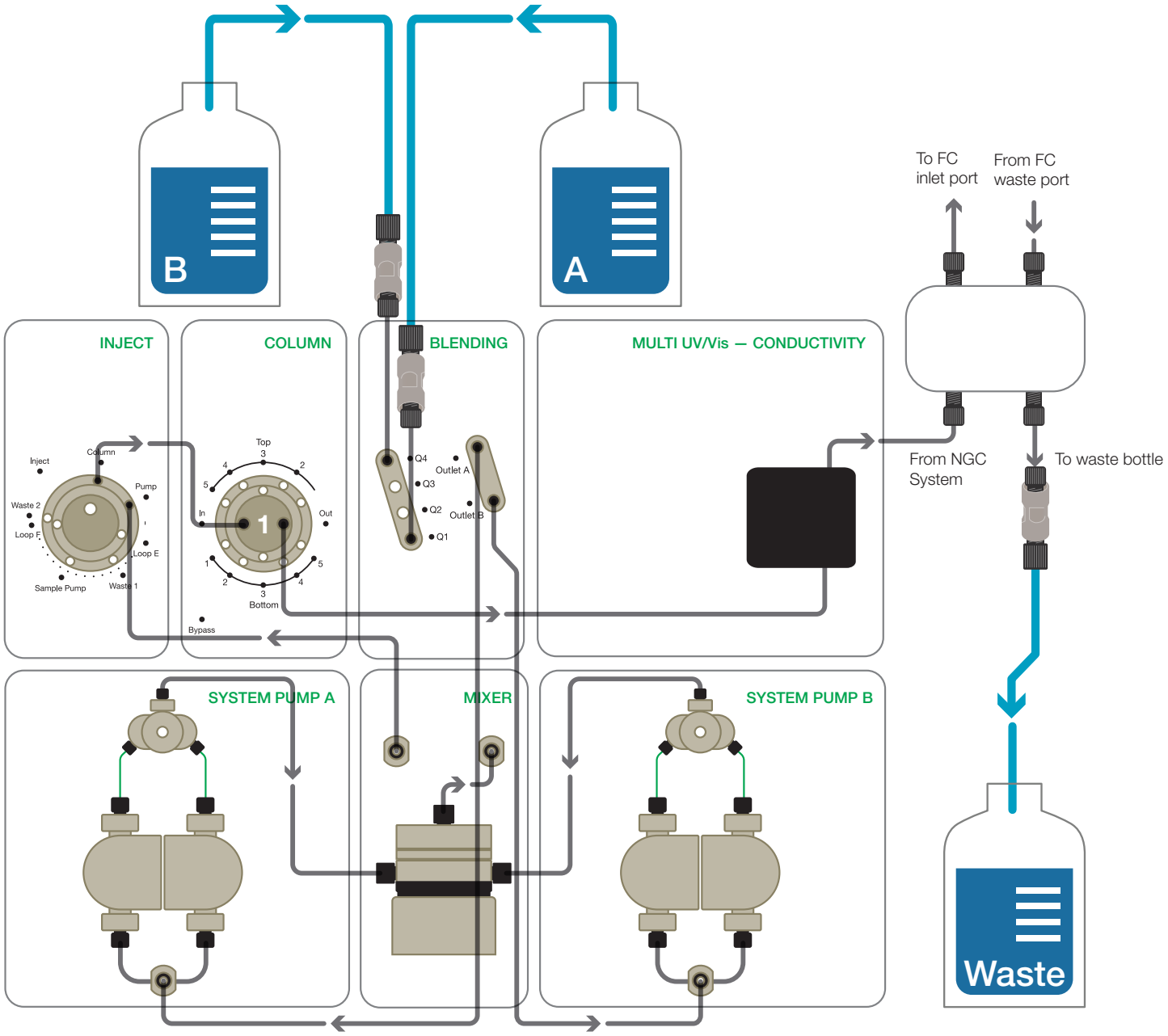
See schematics for examples of high-flow configurations.



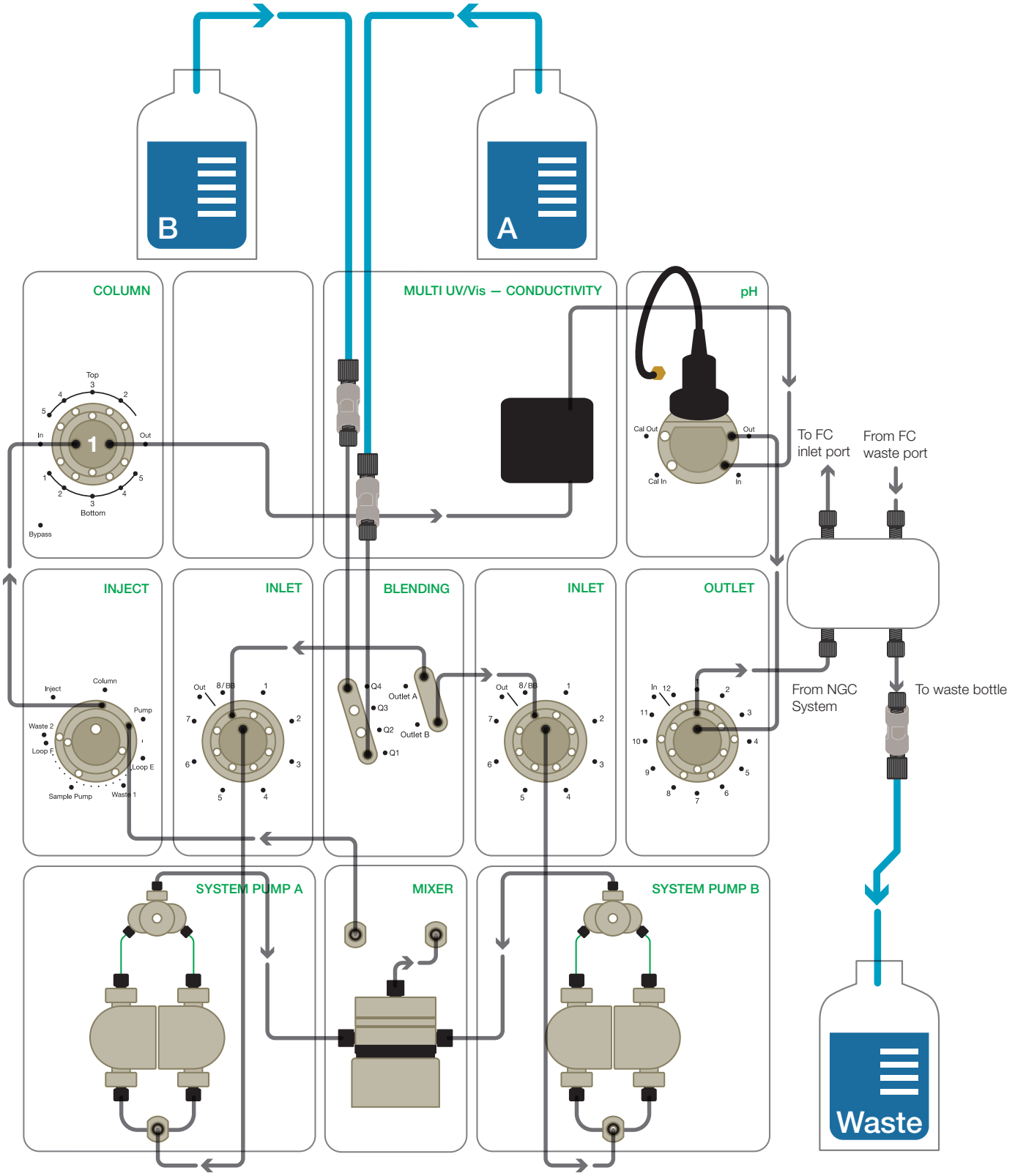
High-flow configuration for flow rates from 40 to 100 ml/min. 3/16" OD x 1.125" ID FEP Tubing with 5/16-24 fitting (—); 1/8" OD x 0.0625" ID FEP Tubing with 1/4-28 fittings (—); 1/16" OD x 0.03" ID green PEEK tubing that comes standard on the system and does not need to be changed out (—). FC, fraction collector; FEP, fluorinated ethylene propylene; ID, inner diameter; OD, outer diameter; PEEK, polyetheretherketone; UV/Vis, ultraviolet and visible light.



High-flow configuration for Buffer Blending Valve for flow rates from 40 to 80 ml/min. 3/16" OD x 1.125" ID FEP Tubing with 5/16-24 fitting (—); 1/8" OD x 0.0625" ID FEP Tubing with 1/4-28 fittings (—); 1/16" OD x 0.03" ID green PEEK tubing that comes standard on the system and does not need to be changed out (—). FC, fraction collector; FEP, fluorinated ethylene propylene; ID, inner diameter; OD, outer diameter; PEEK, polyetheretherketone; UV/Vis, ultraviolet and visible light.



High-flow configuration for Buffer Blending Valve for flow rates up to 200 ml/min. 3/16" OD x 1.125" ID FEP Tubing with 5/16-24 fitting (—); 1/8" OD x 0.0625" ID FEP Tubing with 1/4-28 fittings (—); 1/16" OD x 0.03" ID green PEEK tubing that comes standard on the system and does not need to be changed out (—). FC, fraction collector; FEP, fluorinated ethylene propylene; ID, inner diameter; OD, outer diameter; PEEK, polyetheretherketone; UV/Vis, ultraviolet and visible light.



High-flow configuration for Buffer Blending Valve for flow rates up to 200 ml/min with two buffer inlet valves. 3/16" OD x 1.125" ID FEP Tubing with 5/16-24 fitting (—); 1/8" OD x 0.0625" ID FEP Tubing with 1/4-28 fittings (—); 1/16" OD x 0.03" ID green PEEK tubing that comes standard on the system and does not need to be changed out (—). BB, buffer blending; FC, fraction collector; FEP, fluorinated ethylene propylene; ID, inner diameter; OD, outer diameter; PEEK, polyetheretherketone; UV/Vis, ultraviolet and visible light.

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