

Optimizing the NGC Chromatography System for Cryo-EM Applications

Quick Guide

Bio-Rad NGC Chromatography Systems are well suited to many protein purification applications. Cryogenic electron microscopy (cryo-EM) applications favor chromatography systems with very low overall swept volumes to ensure high-resolution separation and fractionation of small volume samples. This quick guide describes how Bio-Rad NGC Chromatography Systems can be optimized for use in cryo-EM laboratories.

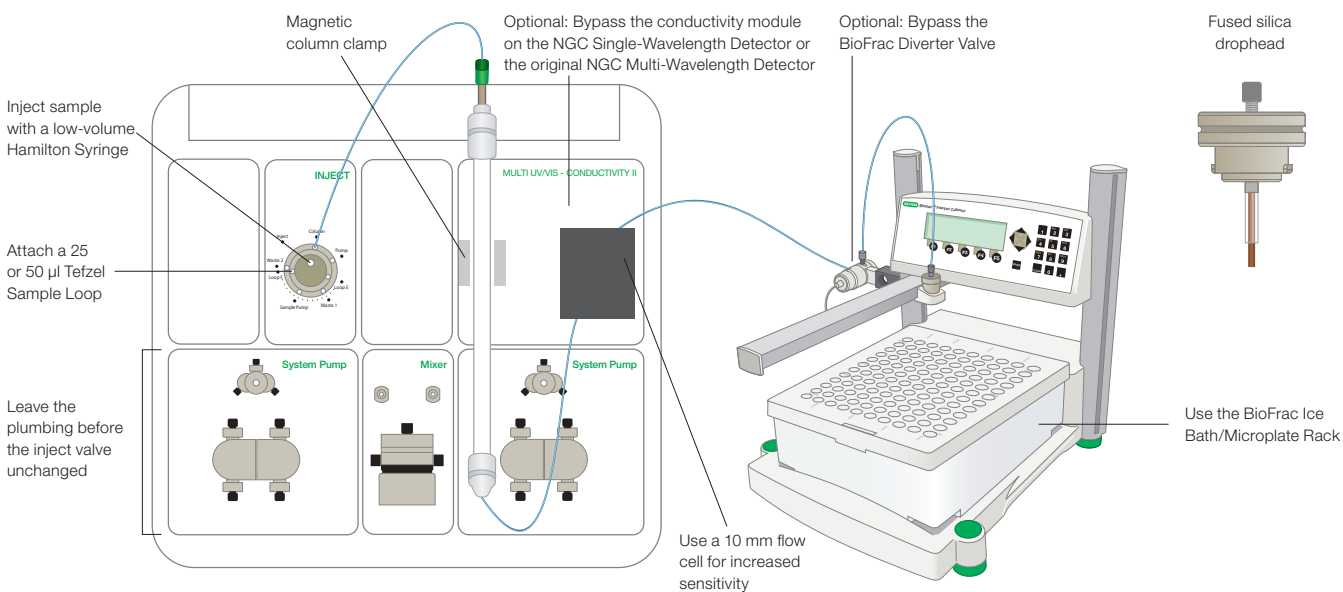
Configuring Your NGC Chromatography System for Cryo-EM Applications

The NGC Quest and Quest Plus Chromatography Systems are ideal for cryo-EM applications because they allow swept volumes to be reduced (by using PEEK Tubing with a narrower inner diameter [ID]) while maintaining flow rates and pressures compatible with the size exclusion chromatography columns commonly used in this application. The system configuration described below minimizes tubing lengths and omits unnecessary components that would only add volume to the flow path. The 10 mm flow cell enhances the sensitivity of the detector to aid in monitoring samples. Using the BioFrac Fraction Collector is also

recommended because the divert valve can be bypassed entirely (if desired) and the drophead can be easily modified with a small piece of fused silica tubing to reduce the drop size to about 8 μ l.

Tubing Configuration

The NGC Chromatography System should be plumbed with narrow-ID PEEK Tubing from the injection port through the drophead nut to minimize the system's total swept volume. Blue PEEK Tubing with an ID of 0.01" is recommended for this application. Table 1 lists other compatible tubing options. When considering which to use, note that total system pressure will increase as tubing ID decreases. Use only the modules essential for your task; if you have an NGC Scout, an NGC Discover, or an NGC Discover Pro Chromatography System, physically bypass the unused modules, plugging them for protection, and operate the system with an accordingly minimized fluidic scheme. The Backpressure Regulator should not be used. Because samples for cryo-EM applications will typically be small (10–50 μ l), use a 25 or 50 μ l Tefzel Sample Loop and a 100 μ l Hamilton Syringe for injection.



Fused Silica Drophead Modification

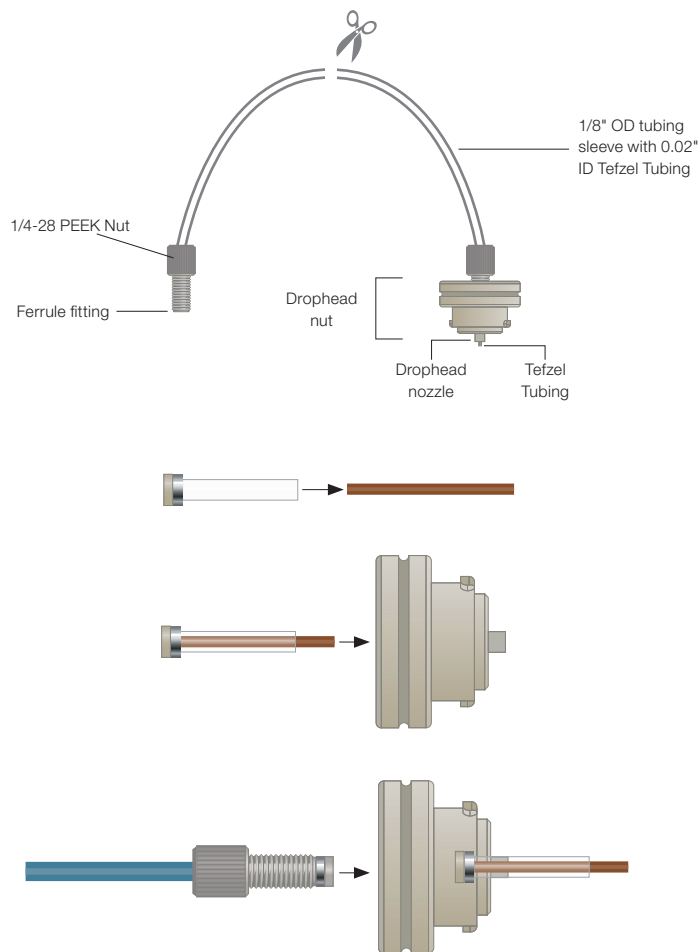
The Biofrac Microplate Drophead Kit uses 0.02" ID Tefzel Tubing and produces 25 µl drops. For smaller drops (7–8 µl), fashion a custom drophead from the Biofrac Microplate Drophead Kit and a piece of fused silica, as described below.

1 Cut the tubing from the drophead kit midway as shown and remove the external 1/8" OD (outer diameter) protective tubing to expose the internal 0.02" ID Tefzel Tubing. Remove and retain the PEEK and drophead nuts from the piece attached to the drophead nut. From the other piece, make another cut 3 cm from the ferrule fitting to obtain a 3 cm piece of 0.02" ID Tefzel Tubing with a connected ferrule fitting.

2 Cut a 4 cm piece of fused silica (orange) and insert into the cut end of the 3 cm piece of 0.02" ID Tefzel Tubing until it is flush with the flat end of the ferrule fitting. The fit will be snug enough to hold the fused silica tubing in place.

3 Insert the fused silica end of the piece made in step 2 into the top of the circular drophead nut until the ferrule is snug at the bottom.

4 Insert the new drophead unit with fused silica into the BioFrac arm (not illustrated) and then connect it to your NGC System by attaching PEEK Tubing (blue) with a 1/4-28 PEEK nut and ferrule fitting to the top of drophead unit.



Delay Volume Calculation

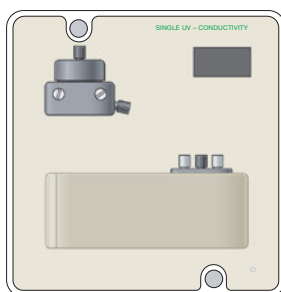
The delay volume of your system configuration can be calculated from the length and specifications of your preferred PEEK Tubing. Measure the total length of PEEK Tubing used to connect the detector module to the drophead nut; use this length to calculate the tubing volume corresponding to your tubing type. See Table 1 for example volume calculations for different PEEK Tubing types. Add the tubing volume to that of your UV/Vis flow cell and any other inline components to obtain the delay volume of your system configuration. Manually enter the delay volume in the system settings for precise fractionation.

Table 1. PEEK Tubing volume calculation.

PEEK Type	ID, in.	ID, mm	Volume, µl/cm	Volume, µl*
Green	0.030	0.76	4.56	342.00
Orange	0.020	0.51	2.03	152.25
Blue	0.010	0.25	0.51	38.25
Yellow	0.007	0.18	0.25	18.75
Purple	0.006	0.15	0.18	13.50
Red	0.005	0.13	0.13	9.75

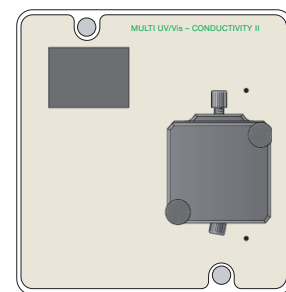
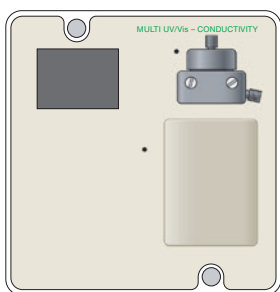
* Volume for 75 cm of PEEK Tubing, the typical length needed to create a flow path from the detector module to the drophead nut. ID, inner diameter.

Additional Inline Component Volumes



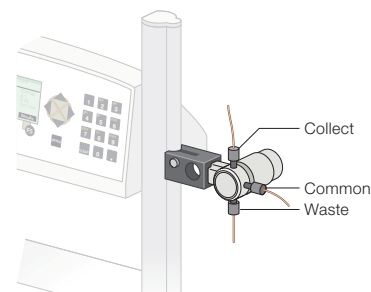
NGC Single- and Multi-Wavelength Detector Modules

10 mm NGC UV Flow Cell — 18 µl
 Conductivity Monitor Flow Cell — 6 µl



NGC Multi-Wavelength Detector II Module

10 mm NGC UV and Conductivity Flow Cell — 24 µl



BioFrac Diverter Valve

12 µl

Recommended Materials for Low-Volume Chromatography Applications

Item	Ordering Information
NGC UV Flow Cell, 10 mm	Bio-Rad Laboratories, Inc. catalog #7885023
NGC UV and Conductivity Flow Cell, 10 mm	Bio-Rad catalog #12012531
Ferrule and Lock Ring	Bio-Rad catalog #7500556
BioFrac Ice Bath/Microplate Rack	Bio-Rad catalog #7410017
BioFrac Microplate Drop Head Kit	Bio-Rad catalog #7410088
Tefzel Plug	Bio-Rad catalog #7500563
25 µl Tefzel Sample Loop	Bio-Rad catalog #7500482
50 µl Tefzel Sample Loop	Bio-Rad catalog #7500483
NGC Column Holder	Bio-Rad catalog #7885038
NGC Dual Column Holder	Bio-Rad catalog #7885057
Purple PEEK Tubing 2 m, ID 0.15 mm, OD 1/16"	Cytiva catalog #18115659
Blue PEEK Tubing 5', ID 0.010", OD 1/16"	IDEX Corporation catalog #1531B
Yellow PEEK Tubing 5', ID 0.007", OD 1/16"	IDEX catalog #1536
Red PEEK Tubing 5', ID 0.005", OD 1/16"	IDEX catalog #1535
Fused Silica Tubing ID 0.15 mm, OD 0.39 mm 2 m, ID 150 µm, OD 360 µm	Thermo Fisher Scientific Inc. catalog #00106-10498 IDEX catalog #FS-115
Fused Silica Carbide Cutter	IDEX catalog #FS-311
100 µL Microliter Syringe Model 710 N Cemented needle, 22s gauge, 2", point style 3	Hamilton Company catalog #80665

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