



Multidimensional (Multi-D) Chromatography on the NGC Chromatography System



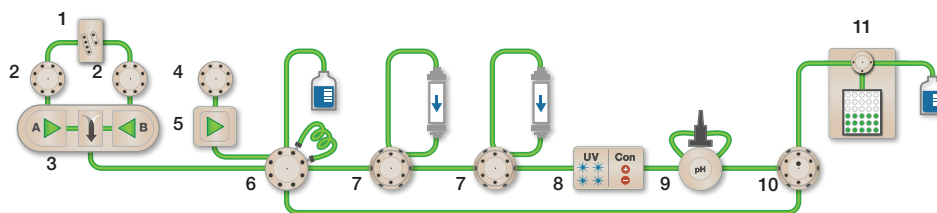
Upon elution from the first column, the sample peak passes through the detector and through the outlet valve to be stored in a clean sample loop.

This purified fraction is then introduced from the sample loop to the second column in the purification workflow.

Introduction

A traditional chromatography purification workflow uses independent columns run sequentially. This type of workflow consists of preparing the system before each column run and collecting, analyzing, and pooling the fractions for each column chemistry utilized. This process requires significant hands-on time with the user present throughout the duration of the workflow. This increased user involvement increases the probability of introducing errors into the workflow and potentially affects the reproducibility of each run. The labor-intensive nature of the workflow also prevents the user from focusing on other tasks.

Multi-D chromatography on the Bio-Rad™ NGC Chromatography System offers several advantages over the traditional chromatography workflow, such as convenience and reproducibility. Though this more automated purification method requires optimized single-purification protocols, it allows continuous multistep purification, providing single push-button functionality to protein purification. This not only allows the user to walk away and focus on other work, but also yields consistent and reproducible purifications with continuous ultraviolet and visible light (UV-Vis) and conductivity monitoring of all elution steps.



Schematic of the NGC Discover 10 Pro Chromatography System (catalog #7880011) with an additional column switching valve (#7884012) illustrates the connectivity between the modules used. 1, buffer blending valve; 2, buffer inlet valves; 3, system pump; 4, sample inlet valve; 5, sample pump; 6, sample injection valve; 7, column switching valves; 8, multi-wavelength detector with integrated conductivity monitor; 9, pH module; 10, outlet valve; 11, BioFrac Fraction Collector.

Components for Automated Multicolumn Chromatography

- Outlet valve — for shuttling the flowthrough or eluted protein from a column to a fraction collector (final fraction collection), secondary storage such as a static loop or large container (application onto another column), or a large open container (flowthrough or column wash collection)
- Column switching valve — 2 CSVs can support up to 10 columns and 1 CSV can be used as a loop valve to hold up to 5 static loops, thus increasing the throughput capability for multiple samples and columns
- Sample pump — allows the application of large sample volumes without contaminating system pumps. It can be used with air sensors to maximize the volume of applied sample
- Sample inlet — for the application of multiple samples and for washing the sample pump with buffer. It can be used with the outlet valve for large-volume elutions or application of the sample from 1 column onto another, and with air sensors to maximize the volume of applied samples
- Buffer inlet — used to change the buffer composition to accommodate the different buffer requirements of each column in the purification workflow

Materials

| Minimum Required NGC System Components | Catalog Number | Components for Multi-D Workflow |
|--|--------------------|--|
| NGC Discover 10 or 100 Pro Chromatography System | 7880011 or 7880012 | Includes 1 column switching valve, 3 inlet valves, 1 sample pump, and 1 outlet valve |
| NGC Column Switching Valve Module, 10 or 100 ml | 7884012 or 7884026 | Minimum of 2 and maximum of 3 for Multi-D applications, with 1 for use as a storage loop valve |
| NGC Inlet Valve Module | 7884006 | 1 for each system pump and up to 2 for the sample pump |
| NGC Outlet Valve Module | 7884013 | Up to 2 per system |
| NGC Sample Pump Module | 7884004 | 1 per system |

Complementary Column Chemistries for Multi-D Purification Applications

| Application | First Column | Second Column | Benefit |
|---|-------------------|----------------------------|--|
| mAb purification | Protein A | Desalting/ buffer exchange | Rapid neutralization of low pH elution buffer |
| mAb purification | Protein A | SEC | Rapid neutralization of low pH elution buffer and aggregation analysis |
| Polyhistidine-tagged protein purification | IMAC | Desalting/ buffer exchange | Buffer exchange to remove imidazole and/or lower ionic strength buffers |
| Protein purification or abundant contaminant protein removal | IEX (flowthrough) | IEX (capture) | Rapid contaminant protein removal; the contaminants bind to the first column while the target protein comes off in the flowthrough |
| Protein purification or abundant contaminant protein removal | IEX (flowthrough) | MM | As above, with enhanced binding and elution selectivity of mixed-mode resin |
| Polishing steps or separation of active from inactive protein forms | IEX | HIC | Eluate from IEX column in high salt binds well to the HIC column |
| Concentration of low-abundance target followed by size analysis | IEX | SEC | Peak separation and analysis |

HIC, hydrophobic interaction chromatography; IEX, ion exchange; IMAC, immobilized metal affinity chromatography; mAb, monoclonal antibody; MM, mixed mode; SEC, size exclusion chromatography.

Resources

Visit bio-rad.com/NGC and these resources for more information:

- Bulletin 6674 — NGC Chromatography Systems Multidimensional (Multi-D) Plumbing Guide
- Bulletin 6694 — Advantages of Multidimensional (Multi-D) Chromatography Using the NGC Chromatography System over Traditional Sequential Chromatography
- Bulletin 6701 — Multidimensional (Multi-D) Chromatography Success Guide
- [youtube.com/watch?v=i4HzxP2tq84](https://www.youtube.com/watch?v=i4HzxP2tq84)

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