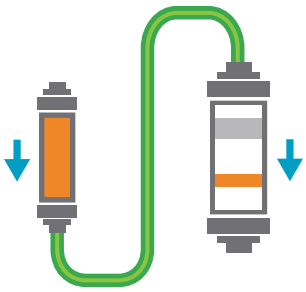

Tandem purification. Upon elution from the first column on the first column switching valve, the sample peak is sent to the top of the second column, which is located on a second column switching valve. Eluate from the second column (or the final column if more than two) in the workflow will be monitored on the ultraviolet and visible light (UV-Vis) detector and fractionated.

Tandem Chromatography on the NGC Chromatography System

Introduction

Tandem chromatography on the Bio-Rad™ NGC Chromatography System offers several advantages over the traditional chromatography workflow, such as convenience and reproducibility. Traditional protein purification workflows typically consist of multiple independent columns run sequentially. This means that the chromatography system preparation and the fraction analysis, fraction pooling, and buffer exchange steps must be performed for each column. 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.

Tandem purification protocols allow for two or more columns to be run, in series, in one purification method in which the eluate from the first column is applied without user intervention to the second column. This can provide a single push-button functionality, allowing the user to walk away and focus on other work without sacrificing consistent and reproducible purifications.

Thorough method optimization of each column in use is essential prior to designing a tandem purification method. A chromatographic readout of the protein of interest will not be observed for the first column, only from the final column in the workflow.

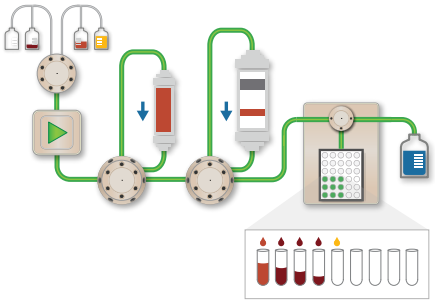

Diagram of a tandem purification method on the NGC Chromatography System.

Components for Tandem Chromatography

- Column switching valve (CSV) — plumbing the columns to 2 CSVs allows the eluate from the first column to be loaded directly onto the second column. The CSVs enable precolumn and delta column pressure monitoring of every column, forward- and reverse-flow elution, and column bypass for system priming or cleaning
- Sample pump — allows the application of large sample volumes without contaminating the system pumps. It can be used with air sensors to maximize the volume of applied sample and with a sample inlet valve for multiple sample loadings
- Sample inlet — for the sequential application of multiple samples and for washing the sample pump with buffer. It can be used with air sensors to maximize the volume of applied samples
- Buffer inlet — used to change the buffer composition to accommodate different buffer requirements for each column. It facilitates cleaning and maintaining the NGC System

Materials

Minimum Required NGC System Components	Catalog Number	Components for Tandem Workflow
NGC Quest 10 Plus Chromatography System or	7880003	1
NGC Quest 100 Plus Chromatography System	7880004	1
NGC Column Switching Valve Module, 10 ml or	7884012	Minimum of 2 and maximum of 3 for tandem applications
NGC Column Switching Valve Module, 100 ml	7884026	Minimum of 2 and maximum of 3 for tandem applications
NGC Inlet Valve Module	7884006	1 for each system pump and up to 2 for the sample pump
NGC Sample Pump Module	7884004	1
NGC 3rd Tier Expansion Frame	7884000	1

Complementary Column Chemistries for Tandem 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 6701 — Multidimensional (Multi-D) Chromatography Success Guide
- youtube.com/watch?v=mxtnj-M-xmM

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