

Configuring the NGC Chromatography System for Tandem Automated IgG Purification with Inline Neutralization

Quick Guide

NGC Chromatography Systems are versatile instruments for protein purification. Here, we describe the optimal NGC System configuration for automated tandem immunoglobulin G (IgG) antibody purification with inline pH neutralization and size exclusion chromatography (SEC). This rapid two-column purification technique minimizes the exposure of antibodies to low pH.

Introduction

The standard workflow for laboratory-scale purification of IgG antibodies involves multiple purification steps. First, the IgG antibody is captured on a Protein A column, washed, eluted by a low pH buffer, and fractionated. The fractions with IgG then need to be neutralized to a physiologically relevant pH for stability and to maintain their native properties. The sample then may need to be concentrated before loading onto an SEC column, or multiple iterative SEC runs may be conducted, if there are loading volume constraints. The SEC column separates the monomeric IgG from aggregates into the preferred final buffer of phosphate buffered saline (PBS).

Alternatively, IgG antibodies can be purified with NGC Systems using a single, automated tandem purification workflow in which antibody eluates are neutralized inline (Figure 1). This workflow utilizes isocratic step-purification via an NGC System Pump (System Pump A) connected to a buffer Inlet Valve. A second NGC Pump (System Pump B) bridging the flow path between two Column Switching Valve Modules (CSV1 and CSV2) enables inline pH neutralization. This quick guide describes the custom NGC System configuration for this alternative approach, including additional components needed (Table 1) and method-creation considerations.

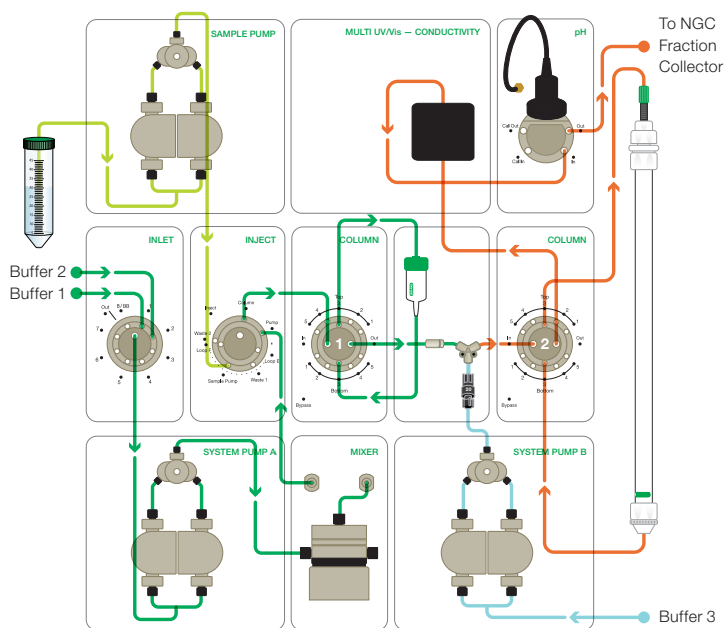


Fig. 1. Flow paths for tandem IgG purification with inline neutralization. The Inlet Valve is primed with two buffers: PBS, pH 7.8 (buffer 1), and 0.1 M glycine, pH 3.0 (buffer 2). System Pump B delivers 1 M Tris, pH 8.0 (buffer 3), for inline pH neutralization. Arrows denote the direction of flow within each path. Sample Pump flow path (■); System Pump A flow path (■); System Pump B flow path (■); neutralization flow path (■). PBS, phosphate buffered saline; UV, ultraviolet; Vis, visible (light).

Sample Pump Flow Path

The Sample Pump flow path (■) is used to load the protein sample. The Sample Pump is plumbed into the Inject Valve to enable selective delivery of the feedstock to the Protein A column on CSV1. If an NGC Sample Pump is not available, a static or dynamic loop could be used to manually load the sample instead.

System Pump A Flow Path

The System Pump A flow path (■) (System Pump A with an NGC Inlet Valve) is utilized for much of the purification workflow. All eight ports of the Inlet Valve are acceptable for use. An example line placement for this inline dilution application is described here.

The NGC Inlet Valve before System Pump A is primed with buffers as follows:

- **Line 1** — PBS, pH 7.8 (for Protein A column equilibration, binding, and washing of IgG sample, and for SEC)
- **Line 2** — 0.1 M glycine, pH 3.0 (for elution of IgG from the Protein A column)

System Pump A is plumbed into the NGC Mixer. The port for System Pump B on the mixer barrel should be closed with a Tefzel Plug from the NGC Fittings Kit.

The NGC Inject Valve is connected to CSV1 with the Protein A column located at position 3. With the SEC column positioned at port 3, CSV2 is set to bypass at this stage to allow for monitoring of the Protein A column.

An inline filter connects the out position of CSV1 (after the Protein A column) to the PEEK Y Connector Assembly. The filter prevents eluted insoluble aggregates from being introduced to the SEC column and helps protect the SEC column from fouling.

System Pump B Flow Path

System Pump B flow path (■) is responsible for delivering neutralizing 1 M Tris, pH 8.0, to the Protein A column eluate as it travels between CSV1 and CSV2 on its way to the SEC column. Instead of being directed to the NGC Mixer, which is used in standard NGC System configurations, System Pump B is connected via PEEK tubing to a PEEK Y Connector with a 20 psi backpressure regulator in between. During inline neutralization, a 4:1 mixture of 0.1 M glycine, pH 3.0 (System Pump A), and 1 M Tris, pH 8.0 (System Pump B), is created by combining flow paths for mixing via the PEEK Y Connector, thereby keeping SEC sample volumes as small as possible.

Neutralized Flow Path

The neutralization flow path (■) begins at the PEEK Y Connector, where the System Pump A flow path (■) combines with the neutralizing buffer driven by the System Pump B flow path (■). As the IgG protein elutes off the Protein A column at pH 3.0, the neutralization buffer is driven into the PEEK Y Connector by System Pump B, combining both flow paths and driving the fluid into CSV2. The neutralized antibody solution is delivered to the SEC column, the CSV1 is placed into bypass, and SEC purification is finished in PBS buffer. As the IgG sample elutes, it is monitored by the NGC Multi-Wavelength Detector II and inline pH monitor before being sent to the fraction collector.

Conclusions

This protocol can be used to obtain up to 50 mg of purified antibody per run. Two-column antibody purification with inline neutralization on the NGC System, as described here, offers scientists a reliable, reproducible, and time-saving solution for laboratory-scale antibody purification.

Table 1. Recommended materials.

Item	Ordering Information
NGC Quest 10/100 Plus Chromatography System	Bio-Rad™ Laboratories, Inc., catalog #7880003, 7880004
NGC 3rd Tier Expansion Frame	Bio-Rad, #7884000
NGC Column Switching Valve Module, 10 ml	Bio-Rad, #7884012
NGC Inlet Valve Module, for System Pump A	Bio-Rad, #7884006
NGC Sample Pump Module	Bio-Rad, #7884004
NGC pH Valve Module	Bio-Rad, #7884011
Tefzel Plug, for unused NGC Column Switching Valve Module ports	Bio-Rad, #7500563
Inline Filter Kit	Bio-Rad, #7500703
PEEK Y Connector Assembly, 1/4–28, 0.06" inner diameter	IDEX Corporation, #P-514
EconoFit UNOsphere SUPrA Column, 1 x 5 ml	Bio-Rad, #12009323

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