LC-MS Sample Preparation: Automated Protein Purification on the Opentrons Flex®

Written by
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INTRODUCTION

Bottom-up mass spectrometry-liquid chromatography (LC-MS) proteomics involves digesting proteins into peptides, separating them by LC, and identifying them using MS. Multiple upstream steps, including isolation and purification, quantification, normalization, protein digestion, and sample clean-up, may be required to prepare samples **(Figure 1)**. Although critical, these steps are time- and labor-intensive for researchers. Automation of these processes can save time, increase throughput, and potentially increase the reproducibility of results. Here, bead-based protein purification of monoclonal antibody (mAb) samples is automated on the Opentrons Flex, resulting in purified samples ready for subsequent steps ahead of LC-MS.

METHODS AND RESULTS

Culture media harvested from three monoclonal antibody (mAb)-secreting HeLa cell cultures, each expressing rabbit IgG and GFP **(Figure 2)**, was added to three separate columns (250 µL per well, 8 wells per column) of a 96-well plate .

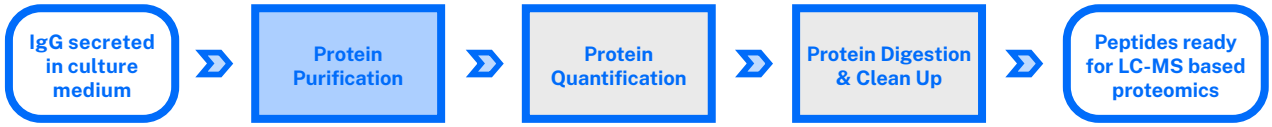


Figure 1. Sample preparation workflow automated on the Opentrons Flex. In this application note, we detail the first step in the process, protein purification. See other application notes in the series for more details on protein quantification and normalization and protein digestion and clean up.

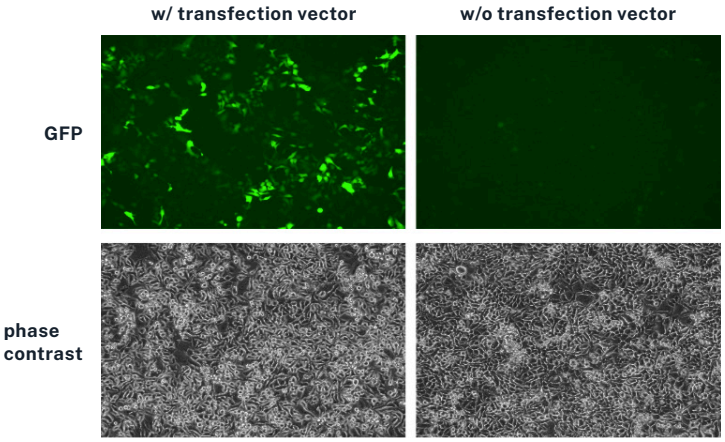
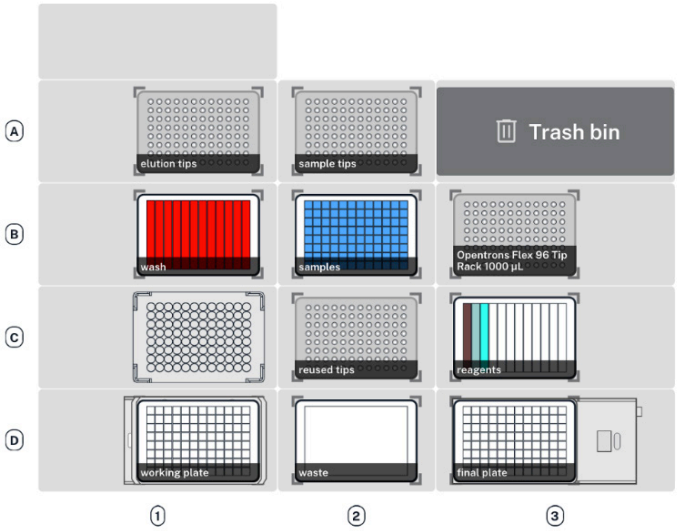


Figure 2. HeLa cells were transfected with a vector carrying rabbit IgG and GFP cDNA for 72 hours. Expression of both was confirmed by visualization of GFP expression with fluorescence microscopy.

Automated protein purification was carried out on the Opentrons Flex **(Figure 3)**. Dynabeads Protein G slurry (50 µL, ThermoFisher Scientific, Waltham, MA) was first rinsed with equilibration buffer (200 µL), then mixed with each sample (200 µL each). The mixture was agitated for two hours on a fully integrated heater-shaker module to capture the target mAb. Next, the Dynabead-protein complex was washed twice with wash buffer (200 µL), and protein was eluted with elution buffer (50 µL).



- Slot D1 - Heater-Shaker Module and Adapter with a NEST 96 Deep Well Plate 2 mL
- Slot D2 - NEST 1 Well Reservoir 290 mL
- Slot D3 - Temperature Module and Aluminum Block with a NEST 96 Deep Well Plate 2 mL (if the final products are NOT immediately subjected to SDS-PAGE)
- Slot C1 - Magnetic Block
- Slot C2 - Opentrons Flex 96 Filter Tip Rack 1000 µL
- Slot C3 - NEST 12 Well Reservoir 15 mL (Bead slurry in Well 1, antibody in Well 2, elution buffer in Well 3)
- Slot B1 - NEST 12 Well Reservoir 15 mL
- Slot B2 - NEST 96 Deep Well Plate 2 mL
- Slot B3 - Opentrons Flex 96 Filter Tip Rack 1000 µL
- Slot A1 - Opentrons Flex 96 Filter Tip Rack 1000 µL
- Slot A2 - Opentrons Flex 96 Filter Tip Rack 1000 µL

Figure 3. Deck setup for an automated protein purification protocol on the Opentrons Flex. Eluates collected from the same cell culture were pooled and subjected to SDS-PAGE and Western blot. The results demonstrated successful target protein enrichment **(Figure 4 upper)** and confirmed the presence of mAb **(Figure 4 lower)**.

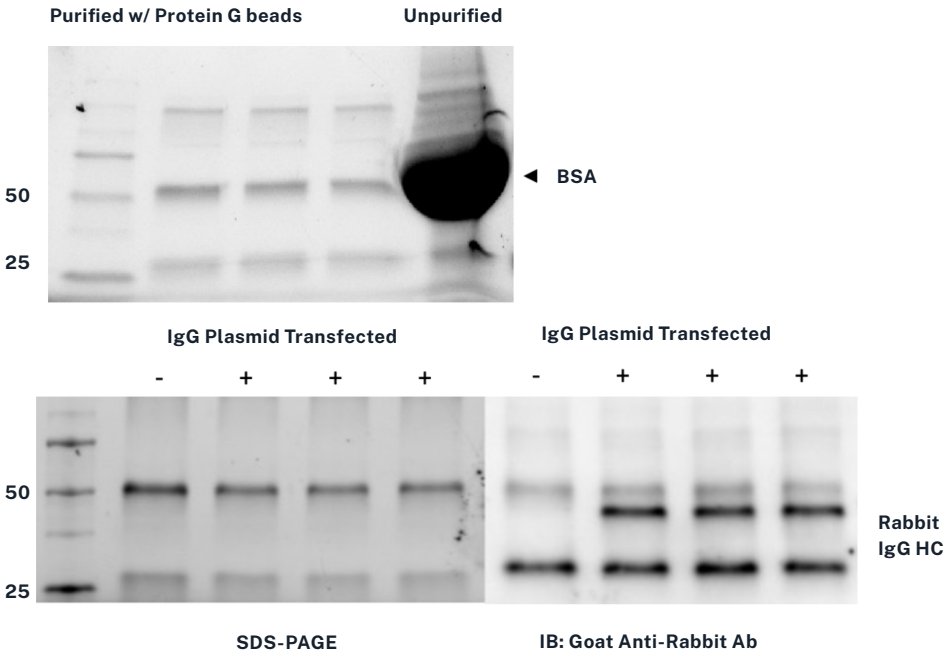


Figure 4. Purified rabbit IgG in each eluate was confirmed by SDS-PAGE and western blot. After purification, the level of non-target proteins was significantly reduced (upper). IRDye® 680RD Goat anti-Rabbit IgG (LI-COR Biosciences, Lincoln, NE) was used to detect the presence of rabbit IgG (lower).

CONCLUSION

The Opentrons Flex and publicly available protocols can be used to automate protein purification, including collecting recombinant monoclonal antibodies obtained from a protein expression system for research or clinical applications. An automated approach offers a convenient solution for routine tasks, leading to reduced labor requirements or allowing for the integration of sophisticated workflows in LC-MS-based proteomic analysis.