LC-MS Sample Preparation: Automated Protein Quantification and Normalization on the Opentrons Flex®

Written by
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INTRODUCTION

Purifying and identifying proteins of interest in a sample is a cornerstone of proteomics research. After isolation, quantification and normalization of total protein provides a baseline ahead of sample digestion and clean-up for liquid chromatography-mass spectrometry (LC-MS) **(Figure 1)**. Automation of the repetitive pipetting in these necessary steps can increase throughput and save researchers valuable time. Here, two protocols for the Opentrons Flex liquid handling robot were used to automate quantification and normalization of a purified monoclonal antibody as part of a complete workflow for bottom-up proteomics.

METHODS AND RESULTS

Two publicly available protocols were used to automate quantification and normalization of monoclonal antibody samples **(Figure 2)**. Six replicates of each purified monoclonal antibody collected from three cell cultures, BSA standards in two-fold dilution, and BCA reagents (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, MA) were manually loaded on the Opentrons Flex platform. The robot performed preparation of BCA working reagent and mixing (200 µL per reaction) with samples or standards (25 µL per reaction), followed by a brief agitation on a fully integrated heater-shaker module and incubation (30 minutes at 37 °C). Absorbance was then measured on deck by a plate reader module at 562 nm. Standard curves were generated, and concentrations of three purified monoclonal antibodies were estimated **(Figure 3)**.

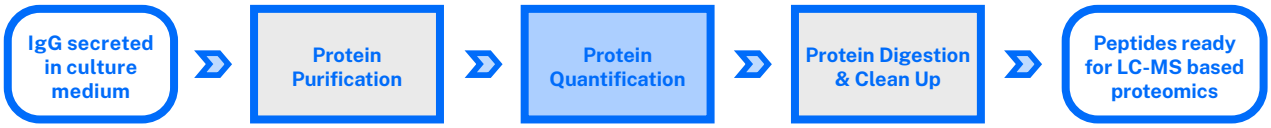
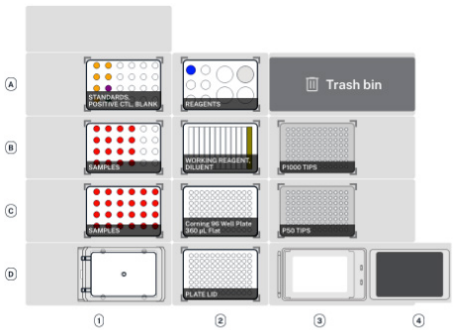
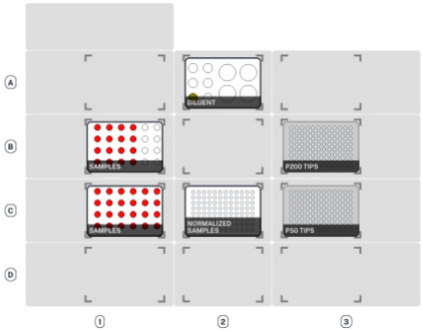


Figure 1. Sample preparation workflow automated on the Opentrons Flex. In this application note, we detail the second step in the process, protein quantification and normalization. See other application notes in the series for more details on protein purification and protein digestion and clean-up.



Protein Quantification and Normalization - Part 1: Pierce BCA Protein Assay

- Slot C2 - Corning 96 Well Plate 360 µL Flat or compatible
- Slot D2 - Plate lid for Corning 96 Well Plate 360 µL Flat or compatible
- Slot D1 - Heater Shaker Module with Universal Flat Heater-Shaker Adapter
- Slot C1 - Opentrons 24 Tube Rack with NEST1.5 mL Snapcap (up to 24 samples) or NEST 96 Deep Well Plate 2 mL
- Slot B1 - Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap (if > 24 samples)
- Slot A1 - Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap or Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt or NEST 96 Deep Well Plate 200 mL
- Slot A2 - Opentrons 10 Tube Rack with NEST 4x50 mL, 6x15 mL Conical
- Slot C3 - Opentrons Flex 96 Filter Tip Rack 50 µL
- Slot B3 - Opentrons Flex 96 Filter Tip Rack 1000 µL
- Slot D3 - Absorbance Plate Reader Module



Protein Quantification and Normalization - Part 2: Normalized Sample Preparation

- Slot C2 - Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt
- Slot C1 - Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap (up to 24 samples) or NEST 96 Deep Well Plate 2 mL
- Slot B1 - Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap (if > 24 samples)
- Slot A2 - Opentrons 10 Tube Rack with NEST 4x50 mL, 6x15 mL Conical
- Slot C3 - Opentrons Flex 96 Filter Tip Rack 50 µL
- Slot B3 - Opentrons Flex 96 Filter Tip Rack 200 µL

Figure 2. Deck setup for automated protein quantification and normalization on the Opentrons Flex.

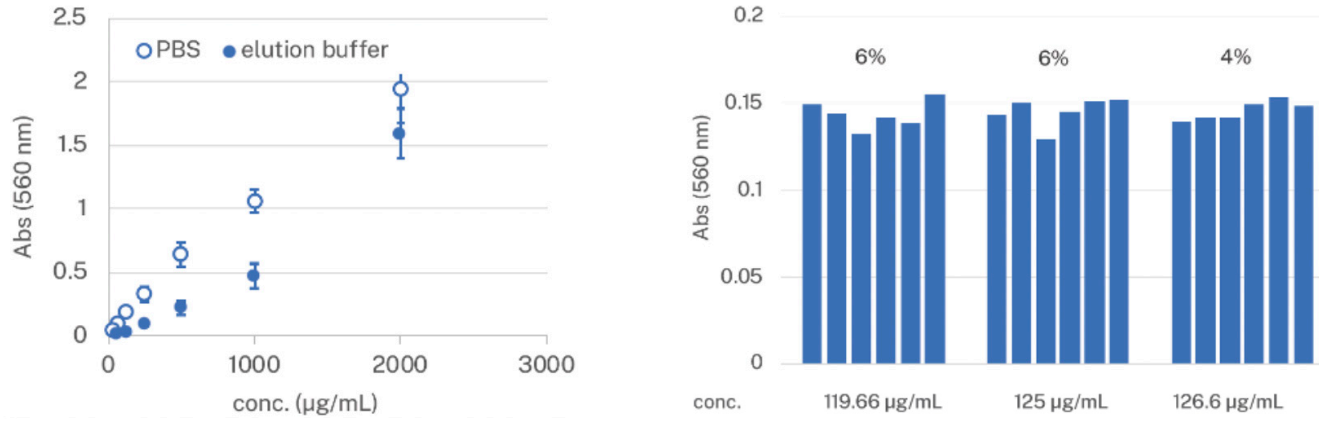


Figure 3. BCA assays were performed to estimate IgG concentrations in purified products by first generating a standard curve with serial two-fold dilutions of BSA in PBS or elution buffer (left, starting concentration: 2000 µg/mL, n=4) and then fitting the absorbance of each sample to the standard curve (right, n=6).

The normalization protocol calculated the volumes for the sample and diluent (100 mM ammonium bicarbonate) and performed liquid pipetting to prepare a new plate with diluted monoclonal antibody (100 ng/µL).

CONCLUSION

The Opentrons Flex and publicly available protocols can be used to automate protein quantification (e.g., BCA assay) and normalization to dilute a target protein to desired concentrations. 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.