Automated Protein Quantification Assays for the Opentrons Flex™

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INTRODUCTION

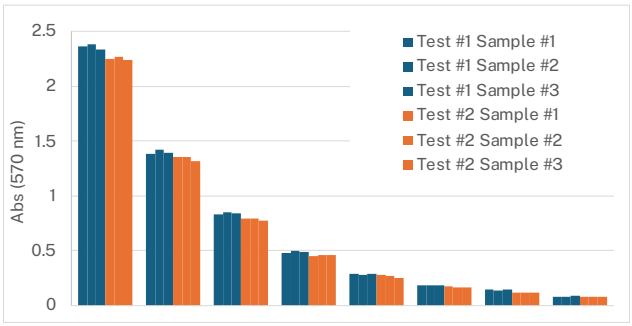
Measuring protein concentrations is an essential part of many routine lab procedures as it is often a prerequisite before subjecting samples to electrophoresis, chromatography, or other molecular biology analysis. Using a robotic liquid handler allows the lab not only to process higher throughput numbers of samples with minimal hands-on time, but also to establish a standardized protocol to consistently generate reliable test results in the long run. Three Opentrons Flex protocols have been developed to respectively perform common protein quantification methods: BCA protein assays, Bradford protein assays and BCA peptide quantification assays (all kits obtained from Thermo Fisher Scientific, Waltham, MA).

METHODS AND RESULTS

The experimental procedures followed a general workflow, wherein the Opentrons Flex was programmed to transfer the working reagent and testing articles into individual wells of a 96-well clear plate with flat bottom. The sample/reagent mixture was agitated for 30 seconds followed by a 30-minute incubation on an Opentrons heater shaker module. The resulting plate was then subjected to analysis utilizing the BioTek Synergy H1 plate reader (Agilent Technologies, Santa Clara, CA). For BCA and Bradford protein assays, serial two-fold dilutions of BSA dissolved in PBS (2000 to 31.25 µg/mL, 7 dilutions) were used as the test articles, and the assay buffer (i.e., PBS) served as blank. For BCA peptide quantification assays, test articles were peptide standards (1000 to 15.625 µg/mL, 7 dilutions) provided alone with the assay kit. For experiments involving BCA protein assays or BCA peptide quantification assays, the robotic system first executed preparation of sufficient working reagent by pipetting and mixing of either Reagent A and B, or Reagent A, B, and C, respectively, upon the number of samples slated for processing. In addition, both BCA-based assays necessitated an incubation at 37°C to facilitate signal development, while Bradford protein assays were maintained at room temperature for the same purpose.
The outcomes confirmed the effectiveness of using Opentrons Flex to perform BSA protein assays (Fig. 1, upper), Bradford protein assays (Fig. 1, middle), and BCA peptide quantification assays (Fig. 1, lower). All these automated protocols demonstrated desirable quality of sample handling and reproducibility (CV <10%). Following the manual arrangement of the labware and reagents, the process was fully automated. The estimated time required for processing 96 samples was less than one hour.

CONCLUSION

The Opentrons Flex protocols streamlines sample processing of protein assays, boosting throughput while minimizing manual intervention and ensuring reliable test outcomes.



µg/mL	2000	1000	500	250	125	62.5	31.25	blank
Test #1	1%	1%	1%	1%	1%	1%	2%	3%
Test #2	1%	1%	1%	0%	5%	3%	1%	1%

µg/mL	2000	1000	500	250	125	62.5	31.25	blank
Test #1	1%	1%	2%	1%	3%	4%	4%	3%
Test #2	1%	1%	1%	2%	2%	2%	4%	2%

µg/mL	1000	500	250	125	62.5	31.25	15.625	blank
Test #1	2%	2%	2%	2%	1%	1%	1%	1%
Test #2	2%	2%	4%	3%	2%	3%	1%	1%

Figure 1. Three sets of 8 test articles (7 BSA dilutions and 1 blank) were processed on the Opentrons Flex for BCA (upper) and Bradford (middle) protein assays, and absorbances measured in duplicate at 570 nm and 600 nm, respectively. Three sets of 7 peptide dilutions and 1 blank were processed for BCA peptide quantification assays (lower), and absorbances measured in duplicate at 450 nm. Two independent experiments of each assay were conducted. Each data point presented on the charts was the mean of duplicate readings from the measurement of each test article, and CV values calculated based on 3 data points at the concentrations as indicated.