

Automated Bicinchoninic Acid (BCA) Assay on the Opentrons Flex®



Written by

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ABSTRACT

Quantitation of total protein is a critical step in many bioanalytical workflows, including proteomics. Automating a quantitation method such as the bicinchoninic acid (BCA) assay can save researchers time while delivering reliable results. Here, an automated BCA protocol was developed for the Opentrons Flex, and its performance was evaluated using four different organ tissues from six Wistar Han rats. The protocol incorporates liquid handling steps with a fully integrated plate reader to automate absorbance measurements in protein standards, quality control (QC) samples, and tissue lysates. Comparisons with a third-party plate reader demonstrated consistency across multiple replicates. Further, total protein quantitation for organ tissue lysates from the fully automated BCA assay on the Flex closely matched manually prepared samples, demonstrating the reliability of the Opentrons Flex for automation of labor-intensive lab applications.

INTRODUCTION

The BCA assay is a colorimetric method for quantifying total protein content of cell or tissue lysates and purified protein products. The assay is performed by mixing diluted protein samples with a BCA working reagent. Under alkaline conditions, peptide bonds and some amino acids can reduce cupric ions (Cu^{+2}) to cuprous cations (Cu^{+1}). Each cuprous cation then forms a purple-colored reaction product (via chelation) with two molecules of BCA. This resulting complex exhibits an absorption at 562 nanometers (nm), enabling researchers to reliably measure absorbance and determine protein concentration of samples. To calculate protein concentration, calibration standards from known concentrations of bovine serum albumin (BSA) are assayed in parallel and used to generate a standard curve.

Reliable protein quantitation using the BCA assay is used for sensitive downstream applications, like protein identification and structural analysis. Incorporating an automated liquid handler to assist with routine pipetting tasks can reduce bench time and minimize human error. Here, an optimized protocol for the Opentrons Flex was developed and tested to automate the BCA assay for protein standards and rat organ tissue lysates, including automated absorbance measurements with a fully integrated plate reader on the Opentrons Flex.

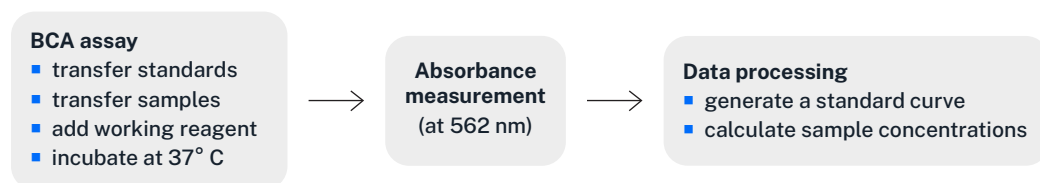
METHODS

Three types of protein samples were prepared using the Opentrons Flex to evaluate the performance of the automated BCA protocol and compare to a manual assay. Eight calibrants (see Figure 2 table below), each in duplicate, ranging in concentration from 117.1 to 2000 $\mu\text{g/mL}$, were prepared with serial dilution of a protein standard (bovine serum albumin [BSA] Pierce™ ampules, 2 mg/mL; ThermoFisher Scientific Pierce BCA protein assay kit, cat. 23227) in lysis buffer: 0.5% Triton X-100 (Fisher Scientific, cat. PI85111) and 1x protease inhibitor cocktail mix (ThermoFisher, cat. 78430), in 50% of tissue protein extraction reagent (ThermoFisher, cat. 78510), diluted in deionized water. An additional three quality control (QC) samples, each in duplicate, were prepared at 1,000 (high concentration; QCH), 500 (middle concentration; QCM) and 250 (low concentration; QCL) $\mu\text{g/mL}$ (also by diluting in lysis buffer). For experimental samples, four organ tissue types (heart, brain, liver, and spleen; $n=6$ per tissue) were dissected from wildtype Wistar Han rats. For both the manual and automated protocols, tissue weighing and homogenization in lysis buffer was performed with an automated homogenizer workstation (Omni LH96) to a concentration of 50 mg/mL (pre-homogenization tissue/lysis buffer w/v). Finally, the Flex prepared a total of 24 rat tissue samples at two dilution levels (10- and 20-fold; 1 replicate each).

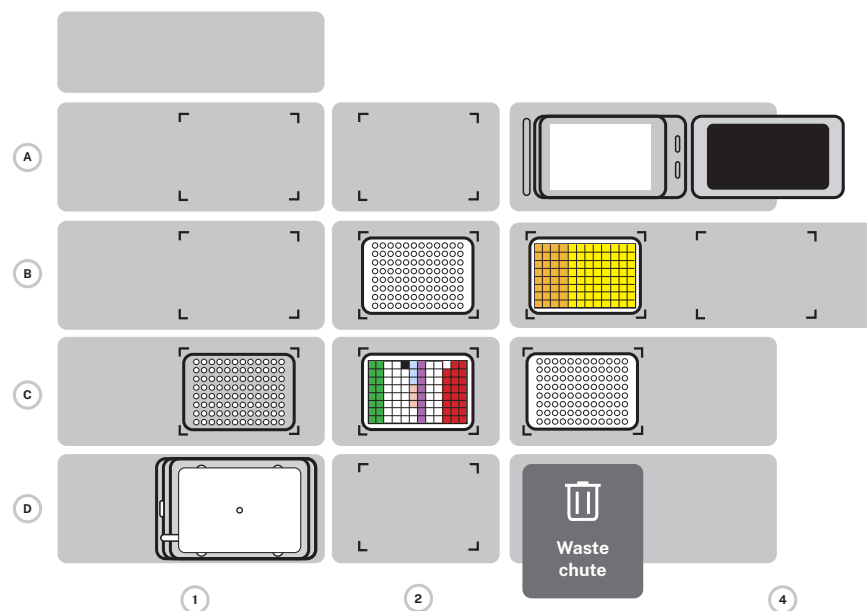
Total protein of calibrants, quality control (QC), and rat tissue samples was quantified by both manual and automated BCA assays. Briefly, 20 μ L of calibrant or quality control/diluted samples were mixed with 180 μ L of BCA working reagent in a 96-well assay plate (ThermoFisher Scientific Pierce™ BCA protein assay kit, cat. 23227) and incubated at 37°C for 30 minutes. Following incubation, absorbance of each plate well was measured at 562 nm with either or both the integrated Opentrons Flex Absorbance Plate Reader or a similar third-party system. Sample and quality control concentrations were calculated from a quadratic calibration curve fit using the calibrant concentrations and absorbance values.

RESULTS

A protocol for the Opentrons Flex was optimized to first dilute standards, quality control (calibrants, QCs) samples, and experimental samples, then transfer dilutions to a 96-well assay plate. Finally, the Flex added BCA reagent to all samples, automated incubation, and measured absorbance. Samples were incubated for 30 minutes at 37°C in an Opentrons Heater-Shaker Module to facilitate chromogenic signal development. Absorbance was measured at 562 nm using the Opentrons Flex Absorbance Plate Reader Module.



Deck View



- A3: Opentrons Flex Absorbance Plate Reader Module
- B2: 96-well plate lid
- B3: 96-deep well plate with samples (columns 1-4)
- C1: Opentrons Flex Tip Rack, 200 μ L
- C2: 2 mL square v-bottom 96-deep well plate with:
 - 1.5 mL BCA reagent per well (column 1)
 - 0.9 mL BCA reagent per well (column 2)
 - 450-600 μ L of BSA standard (2 mg/mL; well A5)
 - 250 μ L lysis buffer per well (B10 -> H10)
 - 1 mL lysis buffer per well (A11 -> H12)
- C3: 96-well BCA assay plate
- D1: Opentrons Heater-Shaker Module

Figure 1. Workflow for the BCA assay, absorbance measurement, and data processing in total protein quantitation (upper). Deck layout for the fully automated BCA assay on the Opentrons Flex (lower).

Eight calibrant samples developed from protein standards were assessed and used to develop a standard curve (**Figure 2**). Absorbance measurements for each calibrant and quality control (QC) sample closely matched across six independent runs, and in a direct comparison between the integrated Opentrons Flex Absorbance Plate Reader and a third-party plate reader (**Figure 3**).

Standard Curve Accuracy & Reproducibility

Calibrant	Concentration (µg/mL)
S1	117.1
S2	175.6
S3	263.4
S4	395.1
S5	592.6
S6	888.9
S7	1333
S8	2000

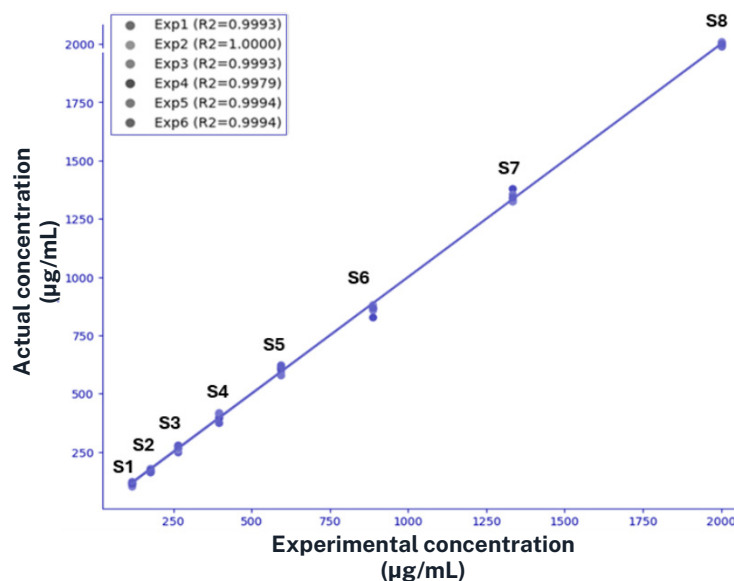


Figure 2. Eight calibrant samples were prepared at multiple concentrations from protein standards (left). Six independent runs were conducted (labeled Exp 1-6), each in duplicate, using the standard scheme of BSA to develop a standard curve (right).

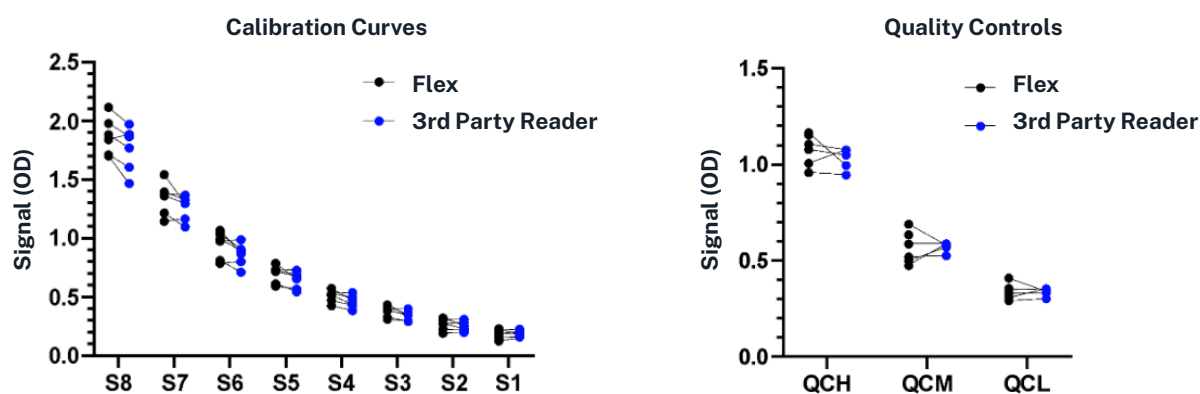


Figure 3. Absorbance measurements of eight calibrants prepared from protein standards (left) and three quality control (QC) samples (right) in duplicate (3 levels; 2 replicates of each: QCH- 1000 µg/mL, QCM- 500 µg/mL, and QCL- 250 µg/mL) were compared between six independent runs. Absorbance measurements were performed at 562 nm on either a third-party plate reader (blue) or the integrated Flex Absorbance Plate Reader Module (black), showing consistency in linearity and signal detection.

Total protein quantification of rat organ tissue lysates (from brain, heart, liver, and spleen) was performed to compare the manual and automated BCA assay protocols. Calculated sample concentrations ($\mu\text{g/mL}$) from a fully automated BCA assay on the Opentrons Flex were in agreement with those measured using the manual BCA protocol across four organ types and six tissue samples of each (**Figure 4**).

Comparison of Sample Quantification Between Flex BCA Protocol and Manual BCA

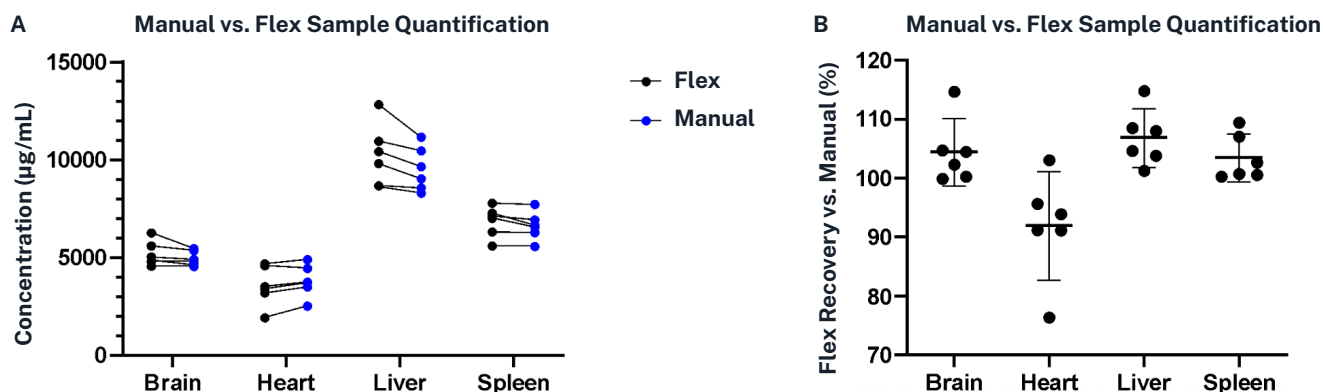


Figure 4. Total protein quantitation was completed for four rat tissue organ types (brain, heart, liver, and spleen) over six tissue samples. Total protein concentrations ($\mu\text{g/mL}$) were measured to compare the manual BCA assay protocol to the automated protocol. Error bars (B) show 95% confidence intervals of the population mean.

CONCLUSION

The BCA assay is a widely used method for total protein quantitation, but can be time-consuming and labor-intensive for researchers. A fully automated BCA assay protocol was optimized for the Opentrons Flex and applied to protein standards, quality control samples, and unknown rat tissue lysates. The absorbance measurements and calculated protein concentrations from the fully integrated Opentrons Flex Absorbance Plate Reader Module were consistent with those from a third-party plate reader, allowing researchers to automate additional steps of their protocols while maintaining assay robustness and reliability. Moreover, the automated Flex BCA assay protocol significantly reduced analyst hands-on time by at least 50%.

Taken together, these results demonstrate the precision and reliability of the fully automated protein quantitation protocol, highlighting the effectiveness of utilizing the Opentrons Flex for standard laboratory applications.