Miniaturization and improved throughput of the BCA protein concentration determination method

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Introduction

Determining total protein concentration is an accepted means of comparing and standardizing biological samples. The utility of protein concentration assessment extends to high-throughput screening platforms including pro- teomic and genomic applications. Ideally the protein concentration determination should also be amenable to high throughput. Most protein determination assays use absorbance measurement detection which are difficult to minimize for higher throughput. Previously, it has been reported that colorimetric assays, including the bicinchoninic acid (BCA) protein assay, can be performed in white plates using fluorescence detection. The method exploits the inherent fluorescence of white plates. In presence of an absorbing solution, the inherent fluorescence is quenched and the decrease in fluorescent signal can be used to measure colorimetric assays. Our results indicate that the miniaturized BCA assay accurately quantifies protein in all plate formats tested, to conserve valuable samples and decrease the amount of reagent. Furthermore, a higher throughput can be achieved in protein concentration determination for subsequent analyses.

Challenge
- Miniaturization of absorbance assays typically meets with limited success
- The short pathlength of low volume samples contributes to noisy data in absorbance assays

Approach to solution
- Miniaturization based on an epifluorescence (fluorescent readout) has been previously successful
- White plates from different manufacturers were tested to miniaturize a BCA assay up to 1536 well format

Materials & Methods
- Pierce™ BCA Protein Assay Kit and Pre-Diluted Protein Standards: Bovine Serum Albumin (BSA) Set (Thermo Scientific)
- White, low volume 384 well plates (Greiner/Corning)
- White, 1536 well plates (Labcyte)
- CLARIOstar microplate reader (BMG LABTECH)

Spectral scanning

Empty wells and wells containing prepared BCA reagent with or without varying concentrations of BSA were added to each plate type. These were scanned with the CLARIOstar using the following settings:

**Excitation bandwidth (nm)**

- Greiner ex: 435-15
- Corning ex: 430-15
- Labcyte ex: 435-15

**Emission bandwidth (nm)**

- Greiner em: 562-20
- Corning em: 545-20
- Labcyte em: 562-20

**Emission wavelength (nm)**

- Greiner em: 562  655  630
- Corning em: 560  655  630
- Labcyte em: 562  655  630

**Excitation wavelength (nm)**

- Greiner ex: 435  475  525  575  625
- Corning ex: 430  470  520  570  620
- Labcyte ex: 435  475  525  575  625

Fluorescent detection of BCA Assay

Replicates of each BCA concentration were added to the plates. A volume of 2 µl was used for 384 well plates and a volume of 1.5 µl was used for 1536 well plates. Prepared BCA reagent was subsequently added. A volume of 10 µl was used in 384 well plates and 7.5 µl in 1536 well plates. The plates were read on the CLARIOstar with the settings indicated below:

**Excitation wavelength (nm)**

- Greiner ex: 435-15
- Corning ex: 430-15
- Labcyte ex: 435-15

**Emission wavelength (nm)**

- Greiner Em: 562-20
- Corning Em: 565-20
- Labcyte Em: 562-20

Data were transformed to enable analysis with a linear regression fit:

\[
\text{OD}_{562} = -\log \left( \frac{F}{F_0} \right)
\]

\[F = \text{fluorescence}, \quad F_0 = \text{buffer blank}\]

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