Comparison of Different Photometric Methods to Measure Protein Concentrations

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Overview

Purpose The purpose of this paper is to evaluate three commonly used photometric protein concentration measurement methods on different sample volumes.

Methods In this study, the direct 280 nm and two of the colorimetric methods: BCA and Thermo Scientific Pierce 660 nm Protein, are compared with two separate proteins. The plate format from 96 to low volume 384 well plates are used.

Results The results show that the Thermo Scientific reagents and microplate instrumentation can reliably and simply be used to determine the protein concentration on several different methods and sample formats from a few micromolars to several thousands of micromolars. It is also shown, that when the sample volume is limited, it is possible to quantify the proteins on a microplate even from volumes down to 20 µl without compromising the performance.

Introduction

Direct protein 280 nm

The direct method is based on the absorption of light in the amino acids containing aromatic side chains. Those are the primary reason for the absorbance peak at 280 nm. The protein concentration can be calculated from the formula: 

\[ A = c \cdot L \cdot \varepsilon \]

As always in photometric assays, the pathlength is an important factor in the assay performance. For the direct method, the theoretical linear assay range can be calculated from the instrument parameters, i.e. instrument precision \( \varepsilon \) – instrument linear range / l. However this value is theoretical, and in normal assay environment it can never be reached.

660 nm protein assay

The Pierce 660 nm Protein Assay is based on a proprietary dye-metal complex that binds to proteins in an acidic solution. Upon binding, the reddish dye-metal complex turns green, resulting in an absorbance shift measurable at 660 nm. The Pierce 660 nm Protein Assay gives even more linear response than the Bradford method, and it is compatible with high concentrations of most detergents and reducing agents. The assay is also fast to perform – the incubation takes only 5 minutes.

BCA

In the BCA protein assay, proteins reduce Cu²⁺ to Cu⁺ in an alkaline medium. The BCA (bicinchoninic) molecule chelates the Cu⁺ ion, which results in a purple product with a strong absorbance at 562 nm. As a method the BCA is less prone to interference than the Bradford method and is more sensitive.

Materials

Reagents Thermo Scientific Pierce Bovine Gamma Globulin Standard (BSG), 2 mg/ml (Cat no 22312)
Thermo Scientific Pierce Albumin Standard (BSA), 2 mg/ml (Cat no 22310)
Thermo Scientific Pierce™ BCA™ Protein assay kit (Cat no 23237)
Thermo Scientific Pierce™ 660 Protein assay kit (Cat no 22650)
Plates Greiner Bio-one UV-Star 96 well plate (Cat no 655801) (Normal 96)
Greiner Bio-one UV-Star 96 well half area plate (Cat no 657801) (Low volume 96)
Greiner Bio-one UV-Star 384 well plate (Cat no 781001) (Normal 384)
Greiner Bio-one µClear 384 well plate (Cat no 78119300) (Low volume 384)

Instruments Thermo Scientific Multiskan GO microplate spectrophotometer (Cat no 51112500)
Thermo Scientific EMISS. Incubator/Shaker (Cat no 51112250)

Methods

The name BSA & BGG standard & sample series were pipetted to normal and small volume UV 96 and 384 plates and measured according to assay instructions.

Direct protein 280 nm

BSA and BGG sample series (8 dilutions each in 2 parallels in plate) were pipetted to normal & low volume 96 & 384 well plates to find out the characteristics in different plate formats.

Sample volumes and the corresponding approximate pathlengths:

<table>
<thead>
<tr>
<th>Volume (µl)</th>
<th>Pathlength (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4.1</td>
</tr>
<tr>
<td>20</td>
<td>0.83</td>
</tr>
</tbody>
</table>

The 384 well plates were centrifuged for 1 minute at 1000 rpm. The absorbances were measured with Multiskan GO at 280 nm using pathlength correction option of the SkanIt software.

Extinction coefficients used were 0.86 for BSA and 1.36 for BGG.

Figure 1. Pathlength correction in SkanIt software for Multiskan GO

BCA Protein assay

BSA and BGG standard and sample series (8 dilutions each in 2 parallels in plate) were measured in normal & low volume 96 & 384 well plates. The samples were diluted 1:16 with BCA Working reagent.

Table 1: Comparison of different methods and sample volumes

<table>
<thead>
<tr>
<th>Method</th>
<th>Normal 96</th>
<th>Low volume 96</th>
<th>Normal 384</th>
<th>Low volume 384</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>550</td>
<td>1100</td>
<td>1155</td>
<td>1170</td>
</tr>
<tr>
<td>BGG</td>
<td>1051</td>
<td>1080</td>
<td>1060</td>
<td>1100</td>
</tr>
<tr>
<td>BCA</td>
<td>1034</td>
<td>1235</td>
<td>1113</td>
<td>1155</td>
</tr>
</tbody>
</table>

The results gained correlate well with those values. The difference between the plate formats can clearly be seen on the lower end of the concentration area. The low volume 96 well plates give the best results because of the longest pathlength. Even 20 µl or lower can be used as the sample volume on a low volume 384 well plate.

BCA and Pierce 660 nm protein assay

The standard curves of both dye based assays are shown on the figures below for the assay performance.

As a summary the concentrations gained on each method are collected to the tables below. As can be seen from the results, the difference for this type of pure proteins is very small. With a good quality photometer all of the assays and plate formats are usable for most of the further applications.

Results

Direct protein 280 nm

In the table below the pathlength corrected absorbances of each sample are compared to the calculated theoretical absorbance.

![Image](image_url)

The theoretical detection range based on the instrument precision and linear range specifications, 0.003 and 2.5 Abs, respectively, are: BSA 14 µg/ml-3700 µg/ml and BGG 7 µg/ml – 1800 µg/ml.

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B缮 and Pierce 660 nm protein assay

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![Image](image_url)

The standard curves of both dye based assays are shown on the figures below for the assay performance.

For the direct method, the theoretical linear assay range can be calculated from the instrument parameters, i.e. instrument precision / ε.

![Image](image_url)

Even 20 µl or lower can be used as the sample volume on a low volume 384 well plate.

![Image](image_url)

Conclusion

- The method to be used should always be chosen based on several factors: performance need, sample type and volume
- It is important to use good quality instrumentation and plates, and the standards to be used with the dye-based material need to be similar to the unknown protein
- Especially in the direct method the sample volume (pathlength) is critical