Bradford, Lowry and BCA Assay Analysis via Magic 1D Software

INTRODUCTION

Protein quantification is the very first step prior to the Western blotting and any further proteomics researches after purification. The most common used methods for protein quantification include Bradford, Lowry and BCA assay. All of them utilize chemical reactions and generate a colorimetrical change from substrate to product. Take Bradford assay for example, the solution color shifts from brown (substrate) to blue (product), and the absorbance change of the reaction is measured through a spectrophotometer at 595 nm in the traditional method. However, in this article, the color changes of Bradford, Lowry and BCA assays will be observed in a new way through a multi-function imaging system, KETA ML, and the image quantification will be performed by Magic 1D software.

MATERIALS

- KETA ML Imaging system with Magic 1D software(Wealtec)
- Bradford protein assay solution (Bio-Rad)
- BCA protein assay kit (Novagen)
- Lowry protein assay

PROCEDURES

1. Protein samples (Bovine Serum Albumin) were diluted to proper concentrations.
2. Bradford, Lowry and BCA protein quantification assays were performed according to the Technical Bulletin #14.
3. After reaction, all samples were loaded into 96 microtiter plates with the same volume and images were taken by KETA ML imaging system with UV/white light converter plate.
4. The image data were analyzed by the “Microtiter Plate Assay” function in the Magic 1D software.

RESULT

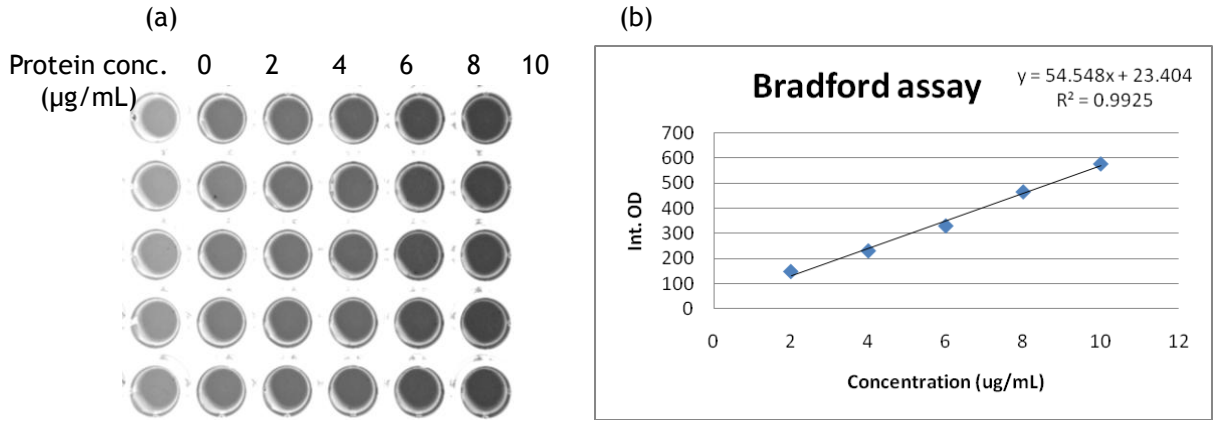

Figure 1. *Bradford assay. (a) Image of the reaction products was taken by KETA ML imaging system with WK101 filter. (b) Standard curve of protein assay was analyzed by Magic 1D software.*

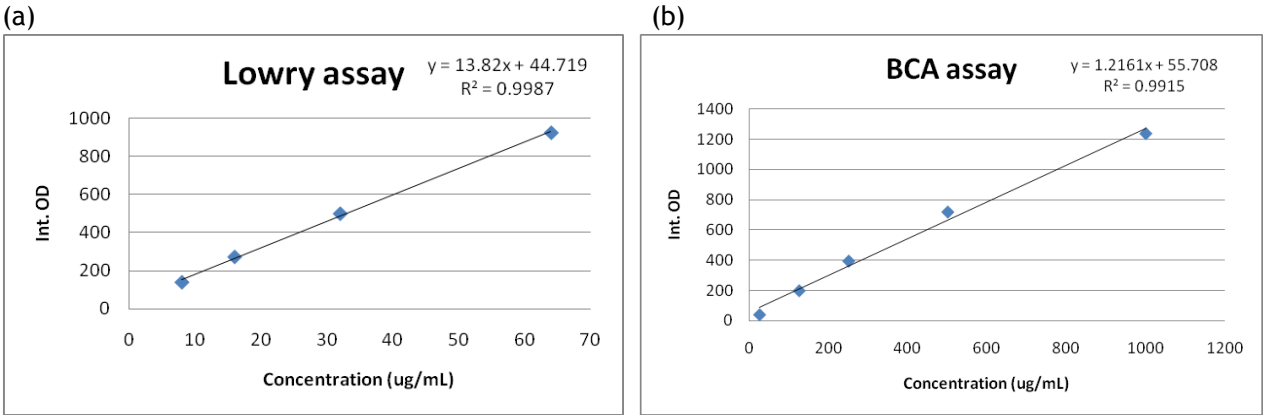

Figure 2. *Standard curve of protein assay calculated by Magic 1D software. (a) Lowry method observed through WK102 filter (b) BCA methods observed through WK104 filter.*

DISCUSSION

KETA ML imaging system equipped with 12 bits CCD provides broad dynamic range with 4096 gray levels which is specifically better for distinguishing on colorimetric. In addition, the software, Magic 1D, offers a powerful analysis tool for calculating the precise quantification of gel image, microtiter plate, colony image and spot analysis. By taking picture of the common-used Bradford, Lowry and BCA protein assay in the

microtiter plate, KETA ML can quantify the protein amount accurately with the standard curve of the R-squared value of 0.99. Besides, while detecting those chromatic samples by an imaging system, using a correct filter also helps to quantify data accurately. For instance, the color of substrate of Bradford assay is mild-brown and interfere the observation of product intensity in a monochromatic CCD system. Adding with WK101 filter can eliminate the interference from the undesirable colored substrates and to distinguish the differences of product intensity more accurately. Lowry and BCA assays, both have the light-colored substrates and less interference on product intensity. Protein quantification even can be performed precisely without a filter requirement, but still, using the filter will be easier for calculate the result.

As the microtiter analysis function in Magic 1D software was so useful, customers can analyze the protein concentration without using a spectrophotometer or ELISA reader. It is a good option for users to quantify their protein samples through taking picture and analyze the spots through KETA series imaging system.

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