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Life Science Spectrophotometer BioSpec™-nano

Application News

# Protein Quantitation Using BioSpec-nano

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## User Benefits

- Micro sample quantities as small as 1 to 2 µL can be measured without using a cell.*1
- The software can calculate the molar absorption coefficient at 280 nm (ε280) by entering the number of tryptophan, tyrosine, and cysteine residues in the amino acid sequence of sample.
- Simply drop the sample and press [Start] to start the measurement. The automatic wiping function makes it easy to successively measure a series of samples.

*1 For samples with weak surface tension, it might not be possible to form droplets that contain a 1 to 2 µL sample quantity.

## ■ Introduction

Protein solutions contain various coexisting substances, such as surfactants, reducing agents, and denaturants, which affect quantitative analysis results. Therefore, it is necessary to select an appropriate method for each sample.

UV absorption (optical density (OD) at 280 nm) is the simplest way to quantitate the total protein concentration using spectrophotometry. This technique, which is based on the 280 nm UV absorption by aromatic amino acids present in proteins, requires no pretreatment and has easy analysis operations. However, absorbance can differ depending on the type of protein, even for the same concentration, and the presence of light-absorbing substances in the same region, such as nucleic acids, prevents quantitation. In contrast, the bicinchoninic acid (BCA) method is a quantitative method that involves small chromogenic differences between different proteins. The method is based on the principle that Cu(I) ions generated in the presence of proteins turn a violet color when they form coordinate bonds with bicinchoninic acid, and is known to offer high sensitivity over a wide concentration range.

Thus, although there are various quantitative methods for proteins, it is required to quantify proteins more simply and in smaller amount in the production of biopharmaceuricals. This article describes an example in which immunoglobulin (IgG) was determined by two methods of OD 280 nm method and BCA method using a BioSpec-nano capable of measuring a micro sample of about 1 to 2 µL.

Fig. 1 BioSpec-nano

## ■ BioSpec-nano

The BioSpec-nano (Fig. 1) can measure micro sample quantities as small as 1 to 2 µL without using a cell (cuvette), making it perfect for measuring valuable biological samples like nucleic acids and proteins.

Measurements can be started by simply dropping the sample at the drop position (target) shown in Fig. 2 and clicking the [Start] button. The wiping function eliminates the need to wipe away samples after each measurement. The BioSpec-nano also provides high reproducibility and measurement accuracy while maintaining high correlation with conventional spectrophotometer systems (using a cell and double-beam configuration). Either a 0.2 mm or 0.7 mm optical path length can be selected*2 depending on the sample concentration, as indicated in Fig. 3. The BioSpec-nano system can be used for many different applications, such as quantitation of nucleic acids, quantitation of proteins, or displaying OD values for specific wavelengths.

*2 An optional 5 mm optical path length is also available (requires 2 mL sample volumes).

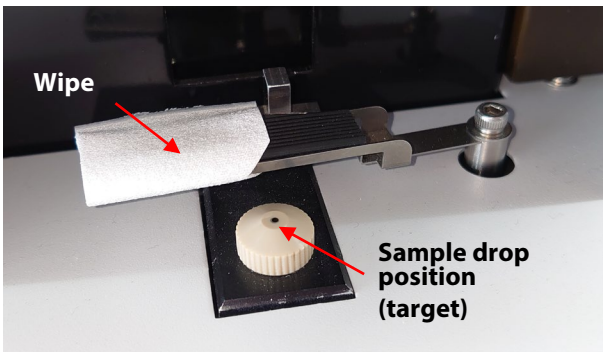



Fig. 2 Sample Drop Position and Automatic Wiping Function

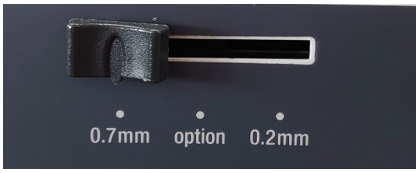



Fig. 3 Optical Path Length Setting

## ■ UV Absorption Method (OD280 nm Method)

By clicking the [Protein Quantitation (OD280)] tab (Fig. 4) and entering the molar absorption coefficient at 280 nm ([ε280 (M-1cm-1)] field) and the molecular weight ([Molecular Weight (MW)] field), the protein concentration is calculated automatically. The ε280 value can also be calculated in software by entering the number of tryptophan, tyrosine, and cysteine residues in the amino acid sequence. The resulting sample concentration values and spectrum can be viewed in the [Detailed View Mode]. Results can be output in either CSV or PDF format.

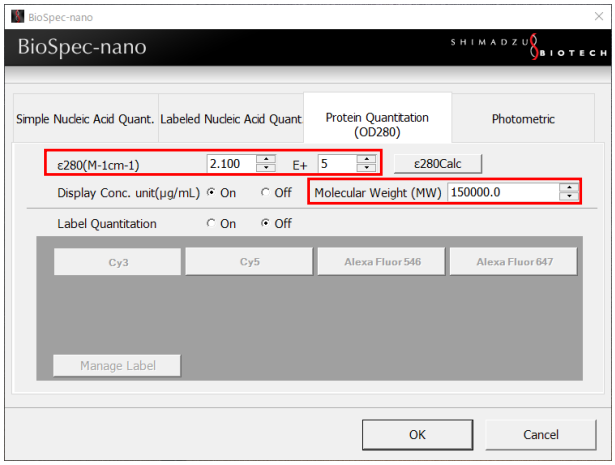


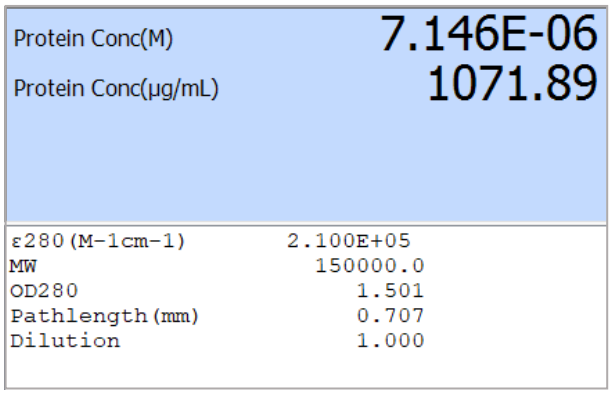

Fig. 4 [Protein Quantitation (OD280)] Tab Page

## ■ Bovine IgG Quantitation Results Using the OD 280 Method

Measurement samples were prepared by diluting bovine immunoglobulin (IgG) to 1,000 μg/mL with phosphate-buffered saline solution.

With the optical path length set to 0.7 mm, the molar absorption coefficient (ε280) set to 2.1 × $10^5$ L/mol·cm, and the molecular weight (MW) set to 150,000, 4 μL sample was dropped onto the target to measure the OD level at 280 nm (with absorbance calculated assuming a 10 mm optical path length).

Measurement results are shown in Fig. 5. The results showed a concentration of about 1,000 μg/mL, as prepared.



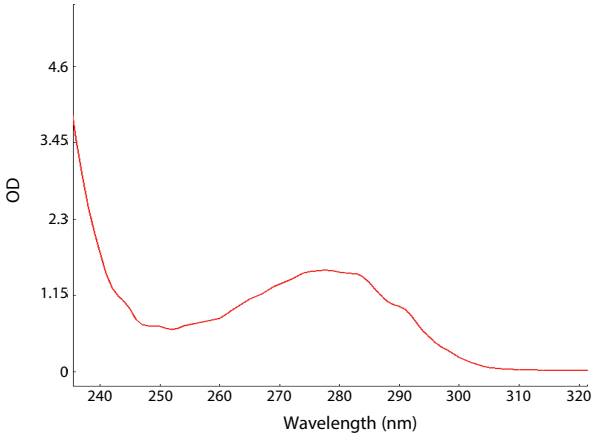




Fig. 5 Bovine IgG Measurement Results with the 0.7 mm Optical Path Length

## ■ BCA Method

The photometric measurement mode can indicate OD values for up to eight wavelengths (Fig. 6). For the BCA method, samples were measured using these photometric measurements.

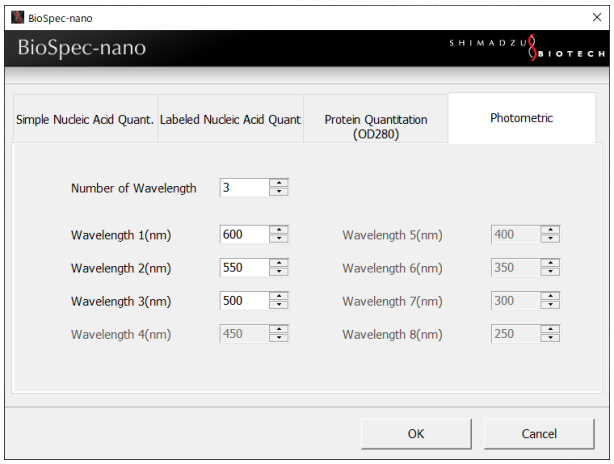



Fig. 6 Photometric Measurement Mode Tab Page

## ■ Quantitation Results of Purified Human IgG Using the BCA Method

1 mL of a commercial human blood plasma was purified to IgG by an anti-IgG antibody column*3, the human IgG concentration was quantitated by the BCA method. 10 µL of purified human IgG solution was diluted 1/2, 1/4, 1/8, 1/16, and 1/32. 100 µL of the reaction solution from the BCA assay kit was added to 5 µL of each diluted solution and the mixtures were incubated for 30 minutes at 37 °C. After setting the optical path length to 0.7 mm, the absorbance of 4 µL of the sample after the reaction at 562 nm was measured in the photometric mode. The standard curve used for quantitation was prepared by measuring bovine serum albumin (BSA) after the same reaction as for the samples. The results indicated good linearity (square of correlation coefficient $R^2$ = 0.9976, Fig. 7). The absorbance values of each dilution of the purified human IgG solution were converted to concentration values using the equation in the standard curve. The concentration values and dilution ratio were plotted (Fig. 8). A linear approximation through the points for dilution ratio that exhibited linearity (1/4 to 1/32) determined that the concentration of the purified human IgG was 2.32 mg/mL.

*3 Refer to Shimadzu Application News 01-00118-EN “Seamless Process from Protein Purification to Evaluation.”

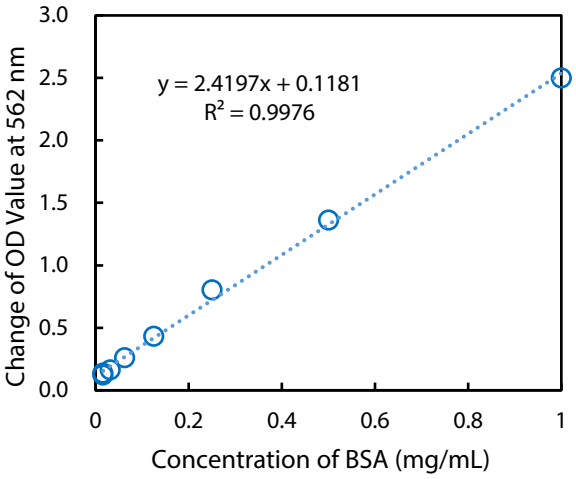



Fig. 7 Standard Curve for Reaction with BSA

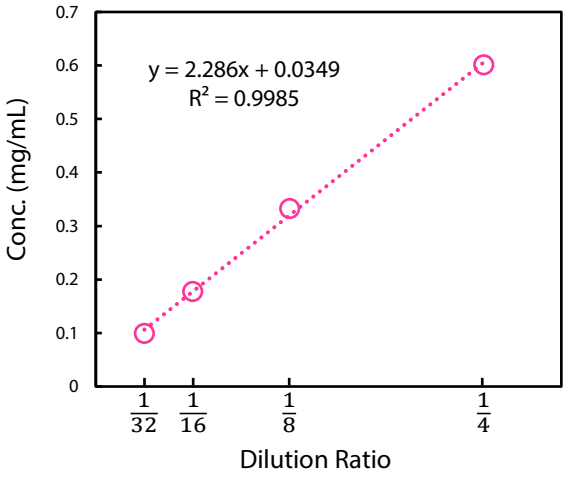



Fig. 8 Measurement Results for Human IgG

## ■ Conclusion

OD 280 nm and BCA methods were used for quantitative analysis of IgG using a BioSpec-nano life science spectrophotometer. Both methods provided excellent quantitative results from small amount of 4 µL sample. These results indicated that accurate measurements can be made by various analytical methods, depending on the type of buffer in which the protein is dissolved, additives such as reducing agents and surfactants, and the characteristics of the sample.
The BioSpec-nano spectrophotometer offers an easy way to quantitatively analyze micro quantities of proteins and is ideally suited for use in biopharmaceutical development and manufacturing applications.



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