

A Two-Way Approach to Easily Determine Protein Concentration

Introduction

The ScanDrop² spectrophotometer system in combination with the FlashSoftPro² software offers high flexibility and adapts to the user's needs. Based on the specific absorption, the concentration of unknown protein samples can be easily determined using two different workflows. For available extinction coefficient(s), the concentration of the target sample can be easily estimated using the formula module. On the other hand, a standard calibration curve is the method of choice if the extinction coefficient is not available or if the sample is rather diluted (lower concentration range). The ScanDrop² spectrophotometer leverages both possibilities, adapting to the user's requirements.

Your Benefits

- Pre-installed measuring modules for easy handling and operation
- Automatic determination of concentrations based on standards
- Advantage through the optional standard line for future applications

Application

Standard concentrations of human immunoglobulin G (IgG) in a range of 0.2 mg/mL up to 6 mg/mL were employed to setup a standard calibration curve using the quantification module (FlashSoftPro²). Within the workflow, the concentration of the targets was automatically determined. In our case, we tested two unknown protein samples. The measurement-series was performed using a Butterfly Cuvette at 0.5 mm path length, thus overcoming the burden of UV-transparent microvolume consumables.

Results

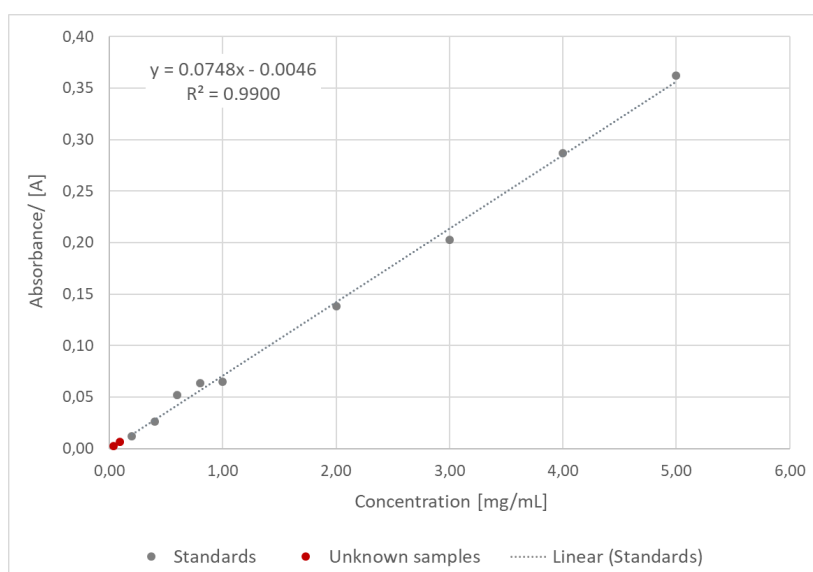


Figure 1: Calibration curve of human IgG . Each calibration point (grey dots), the linear standard curve (grey) and the samples of unknown concentration (red dots) are shown.

Table 1: Overview of measured protein concentrations

Sample	Concentration	Absorbance
Standard 1	6 mg/mL	0.464
Standard 2	5 mg/mL	0.362
Standard 3	4 mg/mL	0.287
Standard 4	3 mg/mL	0.202
Standard 5	2 mg/mL	0.138
Standard 6	1 mg/mL	0.065
Standard 7	0.8 mg/mL	0.064
Standard 8	0.6 mg/mL	0.052
Standard 9	0.4 mg/mL	0.027
Standard 10	0.2 mg/mL	0.012
Sample 1	0.094 mg/mL	0.006
Sample 2	0.037 mg/mL	0.002

For human IgG, the theoretical dynamic detection range of the ScanDrop² and Butterfly Cuvette (0.5 mm path length) is between 0.1 mg/mL and 20 mg/mL.

The quantification module allows automatic setup, storage and reloading of linear calibration curves (see Figure 1 and Table 1).

Additionally, for known sample extinction coefficient (e. g. based on the protein sequence), the Formula module, allows incorporation, storage and reloading of individual equations. A very instructive example is the determination of human IgG protein concentrations based on the extinction coefficient.

Following the second approach (see above) and considering an extinction coefficient value for human IgG of $\epsilon = 1.38^1$, the calculated concentrations (Sample 1 = 0.091 mg/mL and Samples 2 = 0.032 mg/mL) are in a reasonable agreement with the two the previously determined values obtained using the linear absorption range of standard samples (see above).

¹ <https://www.biosyn.com/tew/How-is-Extinction-Coefficient-Determined-for-Proteins.aspx>

Reference: TechNote_ScanDrop²_en_0002_IgG-DRAFT.docx

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