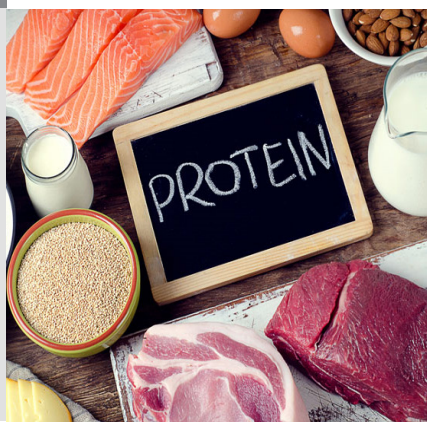
Protein calculations and NanoCuvette™ One



The concentration of proteins in solution can be measured by refractive index. Ready-to-use Excel spreadsheets for protein concentration from refractive index are provided. These spreadsheets are designed to work with data from NanoCuvette™ One. Thereby, proteins can be quantified label-free using a spectrophotometer without UV absorbance or fluorescence at volumes 0.5 µL or 2 mL.

1. Introduction

The concentration of proteins in solution can be obtained by refractive index[1]. In 1935, the linearity is described by Hand[1]:

$$n_{ps} - n_s = a \times C$$

, where n_{ps} is the refractive index of the protein solution, n_s is the refractive index of the solvent, a is the specific refractive increment of the protein and C is the concentration of protein solution in g/ml. Rearranging the above equation, the concentration can be isolated:

$$C = \frac{n_{ps} - n_s}{a}$$

, whereby the concentration of a protein solution can be calculated. Building on this knowledge, ready-to-use Excel spreadsheets are provided on the product webpage as a method to calculate protein concentrations via refractive index.

2. Concentration by specific increment

If the specific refractive increment and the refractive index of the solvent are known, the spreadsheet "Protein concentration 1" can be used. First, select the specific increment and type in the refractive index of the solvent at the top, next type in the measured refractive index and the concentration will be calculated.

The specific refractive increment of protein is approximately 0.190 ml/g at 589 nm for unmodified proteins with molecular weights beyond 10 kDa according to Zhao et al.[2]. This value is widely used for aqueous protein solutions, but may vary depending on solvent[1]. Options for human proteins, zebra fish, yeast, *C.elegans*, *E.coli* and membrane proteins are provided.

3. Concentration by standard curve

The linear relationship between refractive index and protein concentration allows for the definition of a corresponding standard curve. Here, the specific refractive increment is the slope and the refractive index of the solvent is the y-intercept obtained from linear regression.

To this end, the spreadsheet "Protein concentration 2" can be used. First, the refractive index of six different known protein concentrations (of the same protein) needs to be obtained. Next, go to the sub-sheet "Standard curve" and type in the six concentrations and refractive indexes respectively. The sheet will now calculate the specific refractive increment and the refractive index of the solvent. In the sub-sheet "Protein concentrations", the refractive index of the unknown protein solution can be entered and this will calculate the unknown protein concentration.

4. References

1. David B. Hand; The Refractivity of Protein Solutions; 1935; The Journal of Biological Chemistry vol. 108, no. 3

2. Huaying Zhao, Patrick H. Brown, and Peter Schuck; On the Distribution of Protein Refractive Index Increments; 2011; Biophysical Journal Volume 100 May 2011 2309–2317

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