

Methods of Protein Quantification

Technical Note 176

Introduction

DeNovix DS-11 FX Spectrophotometer / Fluorometer Series instruments enable precise absorbance and fluorescence quantification of proteins across a wide dynamic range. The DS-11 FX+ includes 3 measurement modes in one instrument: microvolume absorbance, cuvette absorbance, and fluorescence. Each of the 3 modes enables quantification of different types of protein samples including but not limited to purified protein, cell or tissue extracts, and low concentration samples.

The purpose of this technical document is to describe and compare the methods to quantify proteins using a DeNovix Spectrophotometer / Fluorometer Series instrument.

Protein A280

The Protein A280 app enables rapid and direct quantification of purified protein samples. This app uses the absorbance at 280 nm and Beer's Law to calculate the concentration of protein in the sample. The app is flexible and includes settings for BSA, IgG, E1%, 1A = 1 mg/mL, and MW & Extinction coefficient. Protein A280 can be used in microvolume or cuvette mode, depending on the concentration of the sample. Microvolume mode enables quantification of BSA from 0.04 mg/mL to 1125 mg/mL. Cuvette mode (1 cm) measures BSA concentration as low as 0.002 mg/mL.

Many buffers commonly used with protein samples exhibit significant absorbance around 280 nm. Protein samples suspended in buffers such as RIPA buffers are not appropriate for measuring via Protein A280. If a protein is in an inappropriate buffer, either resuspend the protein in PBS or consider a colorimetric method.

Colorimetrics

Colorimetrics assays are recommended to quantify protein from cell or tissue extracts, unpurified protein, or protein which is suspended in a buffer that absorbs in the UV region. The Colorimetrics app is fully optimized and preprogrammed with the Bradford, BCA, Lowry, and Pierce 660 colorimetrics assays and can be used in either microvolume or cuvette absorbance mode. Other colorimetric assays can be added using the Standard Curve Methods app. Please refer to the assay manufacturers' instructions for concentration ranges and buffer compatibility.

Fluoro Protein and Fluoro Standard Assays

The Fluoro Protein app enables sensitive fluorescence quantification of protein, including unpurified protein. The app is preprogrammed with commercial protein assays. The Fluoro Standard Assays app also enables creation of assays to measure protein. Fluorescently labeled proteins, such as green fluorescent protein (GFP), can be measured directly in fluorescence mode. Refer to Tech Note 170 GFP Quantification Performance Data for more information about measuring GFP concentration.

Summary

The DeNovix Spectrophotometer / Fluorometer Series of instruments enables flexible and accurate quantification of different types of protein samples. DeNovix EasyApps® software is intuitive and easy to use and the Protein A280, Colorimetrics, and Fluoro Protein app are optimized for measuring protein.

