Quantification of labeled proteins

Introduction

In this note, we describe how to use the Protein labeled application on the Lunatic systems. This application determines the concentration of the protein, the amount of dye present in labeled protein samples, and furthermore calculates the degree of labeling (DoL).

App selection

On the Big Lunatic, the Protein labeled application can be found in the "Labeled" column upon selection of "Dyes" in the Sample Type screen (Figure 1). On the Little Lunatic, this application can be found on the applications screen (Figure 2). For proper use of the application, always use the sample solution buffer as blank(s). Aside from sample names, additional user input can be entered:

Dye information: choose a dye from the predefined list or define a new dye with the dye's extinction coefficient [l/(mol.cm)] at max absorbance wavelength [nm], as well as a correction factor at 280 nm.

E1%: define extinction coefficient (default 10)

MW: define molecular weight (g/mol)

Results on screen

The protein concentration is calculated using the A280 value of the baseline corrected UV/Vis spectrum (black curve on the Big Lunatic, white curve on the Little Lunatic), the E1% value and is corrected for dye specific absorbance at 280 nm. When the molecular weight (MW) is entered, protein concentration in pmol/µL and the DoL are also calculated. (Figures 3 and 4)

- Protein concentration (mg/mL)
- **Degree of labeling (DoL)**: dye concentration (pmol/µL) divided by the molar protein concentration, multiplied by 100.
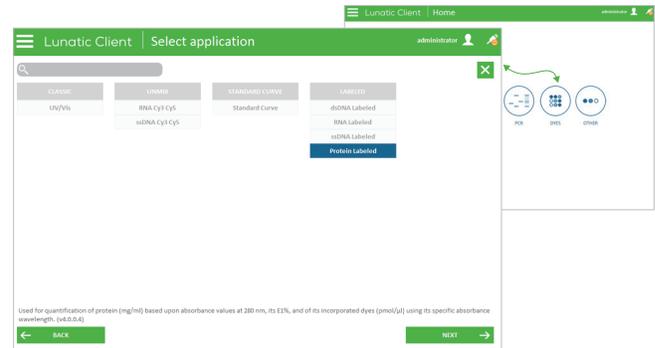

Figure 1: Illustration of the Big Lunatic interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.
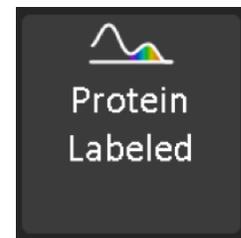

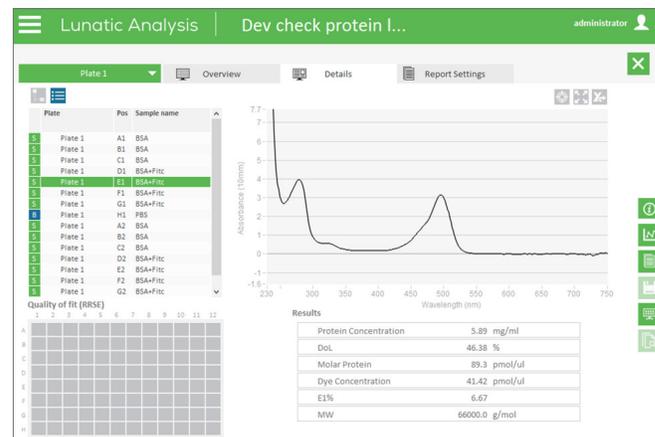Figure 2: App button on the Little Lunatic app selection screen.

Figure 3: Illustration of the Results screen on the Big Lunatic.

- Molar Protein (pmol/µL)

- **Dye concentration (pmol/µL)**: determined using the dye specific wavelength absorbance divided by its extinction coefficient.

- Extinction coefficient (E1%)

- Molecular weight (MW)

Report

A variety of report types are generated: an HTML, XML, TXT and a CSV file are created on both systems. In addition, the Big Lunatic also creates XLSX and PDF report files. On the Little Lunatic fixed report templates are used while the Big Lunatic allows full flexible selection of the content to be reported.

Figure 4: Illustration of the Results screen on the Little Lunatic.

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