Total protein quantification using A280

Introduction

In this note, we describe how to use the classic A280 Protein application on the Lunatic systems. This application is used for quantification of protein (mg/mL) based on the A280 peak height.

App selection

On the Big Lunatic, the classic A280 Protein application can be found under the "Protein" sample type button in the "Classic" column (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 added:

Extinction coefficient (E1%): Define E1% value/values (default 10)

Results on screen

On the Big and Little Lunatic, A280 concentration values are shown in the overview tab and the chip thumbnail view, respectively. For each sample, a more detailed analysis can be found in the Big Lunatic's details tab and below the graph on the Little Lunatic (Figures 3 and 4):

- **A280 concentration**: The baseline corrected UV/Vis spectrum (black curve on the Big Lunatic, white curve on the Little Lunatic) is used to calculate the A280 concentration in mg/mL. This is done using the absorbance values at 280 nm and protein specific E1% percent extinction coefficients (default E1%=10 for unspecified protein mixtures). E1% is the absorbance of a 1% protein solution by mass and has the units g-1 L cm-1. Concentration is calculated as:

$$\frac{(A280 \times 10)}{E1\%}$$

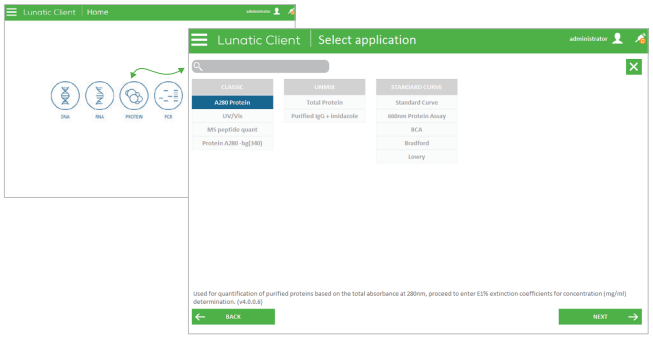

Figure 1: Illustration of the Big Lunatic interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.

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

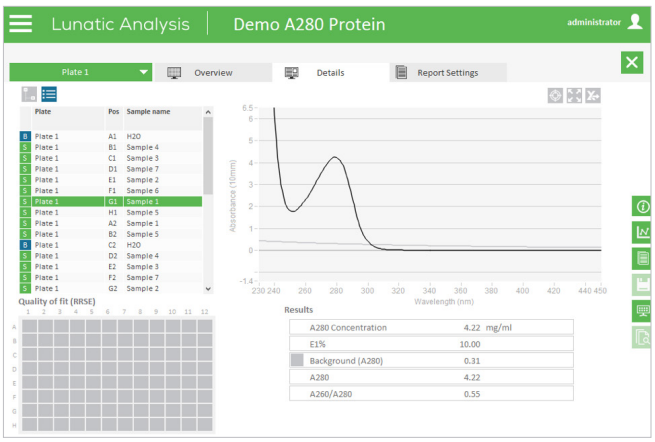

Figure 3: Illustration of the Results screen on the Big Lunatic. In addition to the A280 concentration value, A280 and A260/A280 ratio are displayed.

- **Background (gray)**: sample turbidity profile. The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum.

Also the classical spectrometry data are shown. The A260/A280 ratio is used to determine the purity of the sample. For pure protein samples, the A260/A280 ratio is ~0.6. A horizontal red band on the spectrum indicates saturation when absorbance values have passed the upper limit of detection (200 OD on a high Lunatic Plate or Chip). Values outside the linear range are not reliable.

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.

Case study

In this case study a comparison was made between the NanoDrop 2000 and Big Lunatic. A gravimetric dilution series of a BSA/IgG mixture was measured on both instruments in octuplicate. The measured A280 concentration was plotted against the predetermined target concentration (Figure 5).

The results are very comparable and close to the target value (gray dotted line). In terms of linearity, the R^2 values and equations are also shown, indicating that not only results but also linearity is very similar.

Compatibility

For some protein extraction or purification protocols, detergents are needed to enhance solubility, disrupt cell membranes,etc. In some cases, these detergents can interfere with the self loadability of the Lunatic Chips, resulting in failed measurements (red flagged). In Table 1, the maximum allowed concentrations for most common detergents are listed.

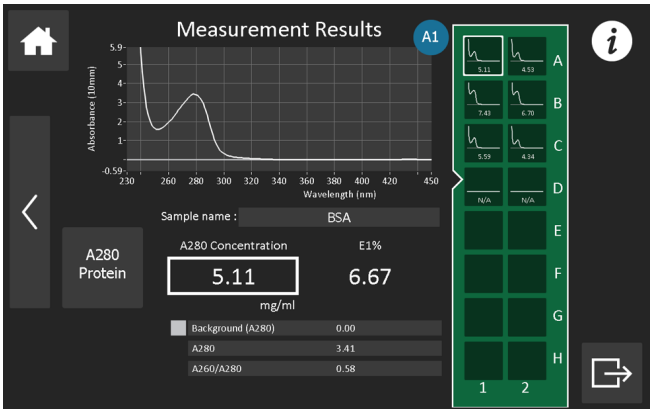

Figure 4: Illustration of the Results screen on the Little Lunatic. In addition to the A280 concentration value, A280 and A260/A280 ratio are displayed.

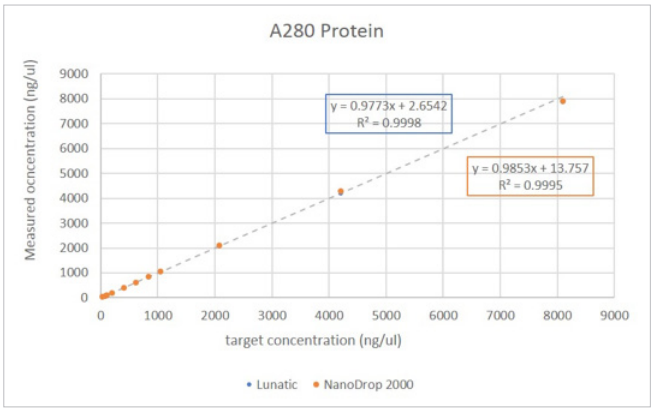

Figure 5: In this graph, octuplicate measurements of a BSA/IgG gravimetric dilution series are plotted against the target values. Measurements on Big Lunatic are shown in blue, NanoDrop 2000 measurements are displayed in red. The gray dotted line represents the y=x line.

Maximal detergent concentration		0 mg/mL BSA	1.5 mg/mL BSA	10 mg/mL BSA
Purification assays	Tween 80	10%	10%	20%
	TritonX-100	0.01%	0.01%	0.01%
	Tween 20	0.10%	2%	2%
Cell lysis	NP40	0.01%	0.01%	0.01%
	SDS	0.5%	0.5%	0.5%
	CHAPS	20%	20%	20%

Table 1: This table shows the maximum detergent concentration where no interference with self-loadability of the Lunatic Chips is found.

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