Quantification of total protein content

Introduction

In this note, we describe how to use the Protein (Lysates) application on the Lunatic systems. This Unmix app is used to analyze the UV/Vis spectral shape of the sample to isolate the fraction of the molecule of interest from co-absorbing entities contributing to the total UV/Vis absorption spectrum. Accurate quantification of the molecule of interest is established using its isolated spectrum fraction. This Unmix app is specifically designed to report an accurate protein concentration and is applicable on purified proteins or complex cell lysates containing an abundant amount of nucleic acids, cell debris and interfering lysis buffer components. Table 1 reports compatibility with commonly used detergents.

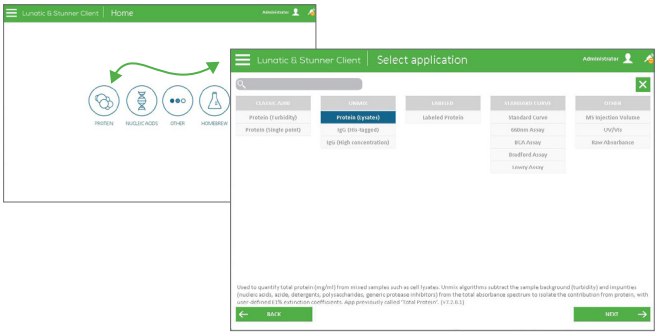

Figure 1: Illustration of the Lunatic "Select application" interface. The image in the back shows the Sample Type screen, whereas the image in the front displays the applications that are available for the selected Sample Type.

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

App selection

On the Lunatic, the Protein (Lysates) application can be found in the "Unmix" column upon selection of "Protein" 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 Unmix applications, always use pure water 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

The Unmix app will analyze the measured UV/Vis spectrum to detect the presence of specific component groups (Figure 3 and 4):

- Protein (green): molecule of interest. The concentration is calculated using the A280 peak value of this profile and the defined E1% value.
- Impurities (blue): non-protein molecules that also absorb in the UV/Vis-region. Components that

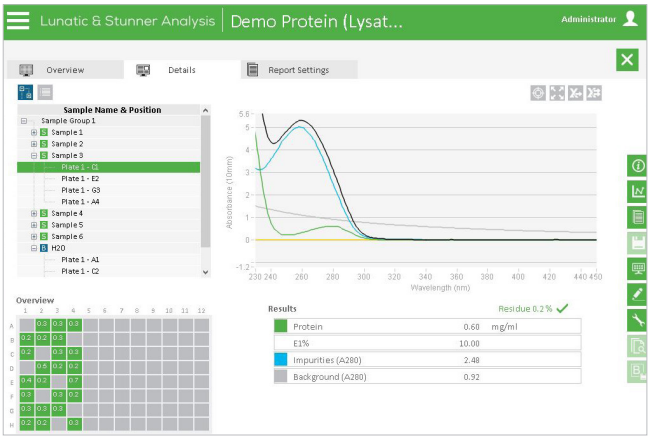

Figure 3: Illustration of the Results screen on the Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values.

are defined as impurities are nucleic acids, azide, protease inhibitor, polysaccharides and NP-40.

- Background (gray): sample turbidity profile. The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum (black curve on the Lunatic, white curve on the Little Lunatic).

The Residue or 'Quality of fit' value (RRSE) is the % of the measured spectrum which could not be annotated, representing the quality of fitting. This parameter is displayed as a yellow curve as well as a percentage value below the graph. A warning sign (red cross) will appear for samples with a residue value above 5% due to (1) too high turbidity of the sample, (2) presence of an unknown chemical, (3) low-concentrated samples.

Report

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

Compatibility

For some protein extraction or purification protocols, detergents are needed to enhance solubility, disrupt cell membranes... 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.

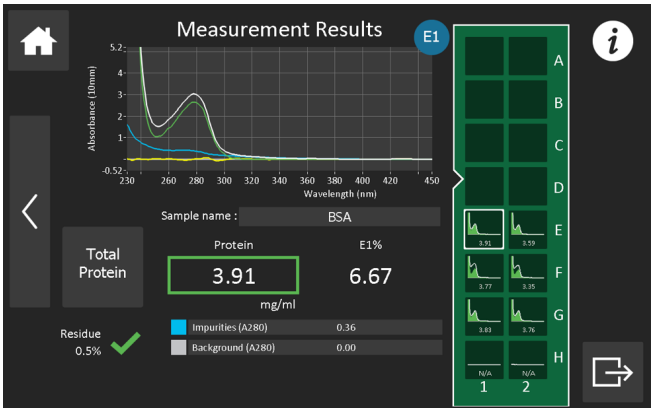

Figure 4: Illustration of the Results screen on the Little Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values.

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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