Quantification of protein samples using colorimetric assays

Introduction

In this note, we describe how to use the colorimetric assay applications on the Lunatic systems. Protein quantification is often done by measuring absorbance at 280 nm, results however might be influenced by buffer or source constituents. An alternative to A280 quantification are colorimetric protein assays. Reagent(s) and proteins are mixed, producing a color change which is a measure for the amount of protein present. Unknown sample concentrations are calculated using a measured standard curve. In this note, applications for 4 commonly used colorimetric protein assays are presented: 660 nm Protein assay, BCA, Bradford and Lowry.

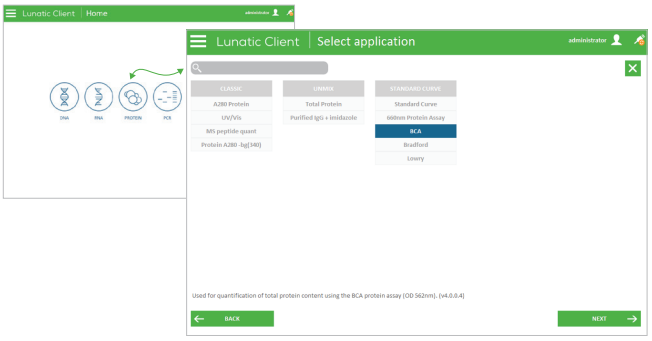

Figure 1: Illustration of the Lunatic interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.

App selection

On Lunatic, colorimetric assay applications can be found in the "Standard curve" column upon selection of "Protein" in the Sample Type screen (Figure 1). On Little Lunatic, this application can be found on the applications screen (Figure 2). Aside from sample names, additional user input will be requested:

New standard curve: define reference values and standard curve type.

Saved standard curve: choose saved standard curve and standard curve type.

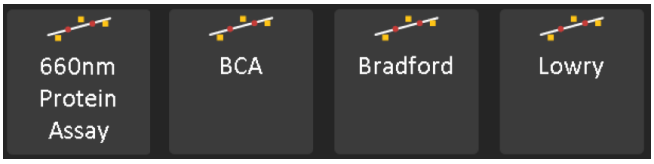

Figure 2: App buttons on the Little Lunatic app selection screen.

Results on screen

On Lunatic, 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 larger system's details tab and below the graph on Little Lunatic (Figures 3 and 4):

- **Concentration** (red square): Calculated in relation to the defined standard curve.

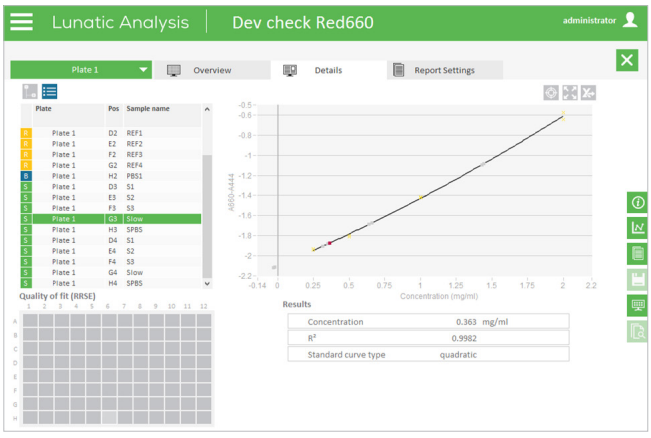

Figure 3: Illustration of the Results screen on the Lunatic. Reference samples (shown as yellow crosses) are used to create a standard curve and concomitant equation to calculate the concentration of protein samples (gray squares, red if selected).

- **R^2**: measure of how good the standard curve results correlate to the fitted regression line (1=perfect fit).
- **Standard curve type**: formula used to fit the standard curve on the reference values.

Report

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

Compatibility

Colorimetric assays are often used to quantify protein lysates. 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.

Additional information

Tables 2 and 3 highlight the different types of standard curves and basic principles of each colorimetric assay respectively.

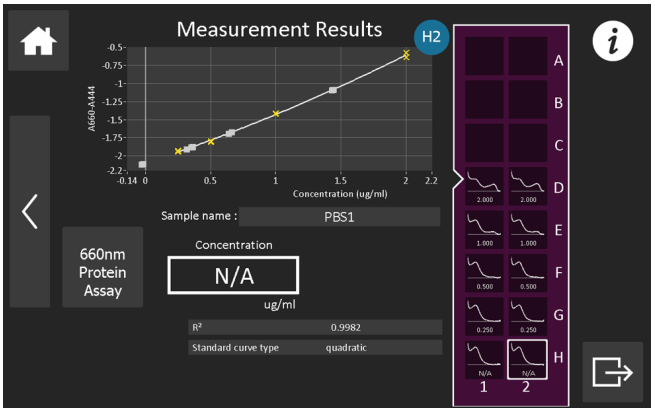

Figure 4: Illustration of the Results screen on the Little Lunatic. Reference samples (shown as yellow crosses) are used to create a standard curve and concomitant equation to calculate the concentration of protein samples (gray squares, red if selected).

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.

Type	Formula	Remarks/use
Proportional	$y = a*x$	Used in the same way as linear, but fitted through zero
Linear	$y = a*x + b$	Line used as best fit between all data points, when values appear to lie on or scattered around a straight line
Point-to-point	-	Linear connection between consecutive points
Quadratic	$y = a*x^2 + b*x + c$	When overall shape of the data points seem to curve, a parabola is fitted
Quadratic (through zero)	$y = a*x^2 + b*x$	Same as normal quadratic but fitted through zero
4 parameter curve fit	$y = \left[\frac{a-d}{1+\{\frac{x}{c}\}^b}\right] + d$	Used when standard curve contains asymptotes (e.g a sigmoidal shape)

Table 2: Different types of standard curves and accompanying formulas. (x= concentration and y= absorbance).

Assay type	Description
BCA	This application was developed using the Pierce BCA Protein Assay Kit (prod #23227). The Bicinchoninic Acid assay is based on BCA/copper chelation resulting in a purple color reaction in presence of proteins.
Bradford	This application was developed using the Bio-Rad Bradford protein assay (prod #500-0001). It's a dye binding assay, in which the Coomassie Brilliant Blue G-250 absorbance maximum shifts upon protein binding.
Modified Lowry	This application was developed using the Pierce Modified Lowry Protein Assay (prod #23240). This assay is based on the formation of a tetradentate copper complex and reduction of the Folin-phenol reagent, producing an intense blue color.
660 nm Protein Assay	This application was developed using the Pierce 660 nm Protein Assay (prod #22660) and Red660 protein assay (G-Biosciences, #786-676). It's based on the binding of a dye-metal complex to proteins in acidic conditions, causing a shift in the dye's absorption maximum, resulting in a green color change.

Table 3: Summary of the basic principles of each colorimetric assay for which an application on Lunatic is present.

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