

DropSense16 App – Colorimetric assays (BCA, Lowry, 660 nm, Bradford)

Quantification of unknown protein samples using Colorimetric assays

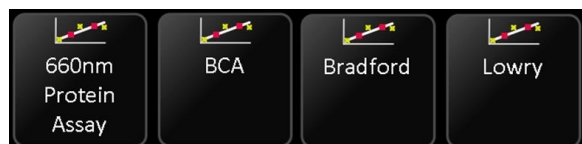


Fig 1. App buttons on the DropSense16 app selection screen

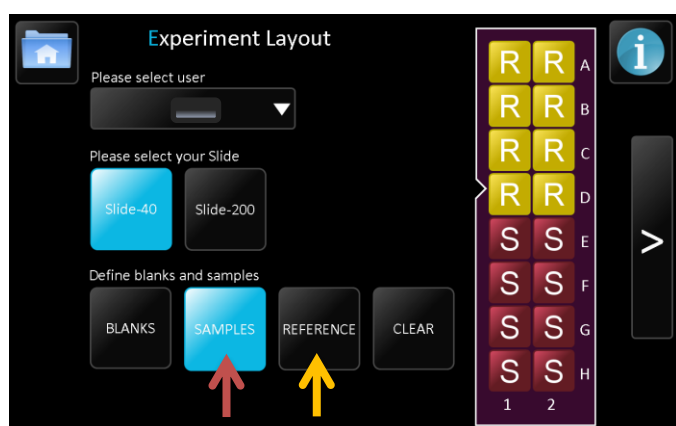


Fig 2. Assign the positions of references and samples

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: * Maximum concentration of detergent not interfering with sample self-loading on Trinean's slides

Introduction

Often protein quantification is done by measuring absorbance at 280 nm, however results 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 colour change which is a measure for the amount of proteins present. Unknown sample concentrations are calculated using a measured standard curve. Apps to measure 4 commonly used colorimetric protein assays are now available on DropSense16.

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 colour.

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 colour change.

Colorimetric assays are often used to quantify protein lysates. Table1 gives an overview of Slide compatibility with detergents.

App description

These apps can be used to quantify unknown sample concentrations by:

- 1] measuring a **new** standard curve (positions have to be defined as references, see figure 2 yellow indications)
- 2] using a previously **saved** standard curve (sample positions are defined as samples, see figure 2 red indications)

It's possible to load references and samples onto the same slide (see figure 2).

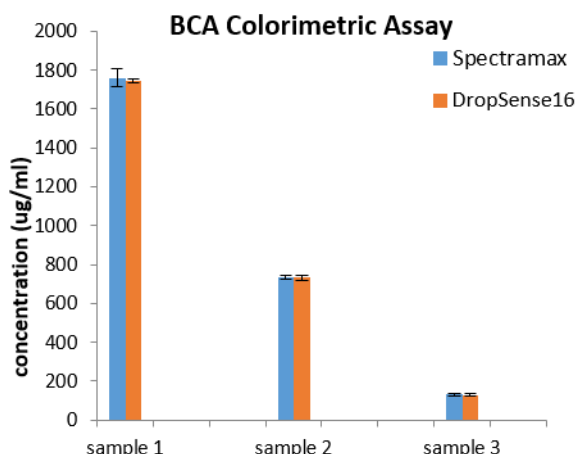


Fig 3. Unknown sample concentrations determined both on DropSense16 and Spectramax

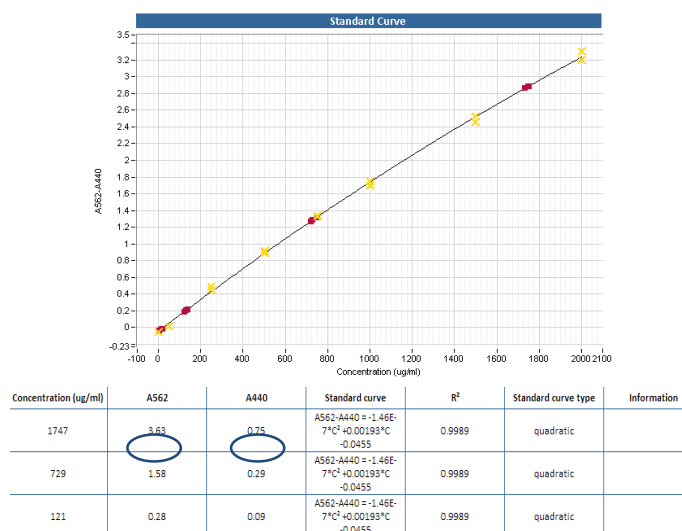


Fig 4. Example of HTML report

Background correction is calculated using a region in the measured spectrum which has the lowest absorbance. This application can be run without measuring a blank solution.

2 wavelength method

Protein quantification in these apps, is done by using a difference in absorbance between 2 wavelengths (derived from the measured UV/VIS spectrum, typical for each colorimetric assay) instead of absorbance at 1 particular wavelength.

Results

Figure 3 shows comparative data for protein samples (concentration range: 125 – 1800 ug/ml) quantified with the Bradford colorimetric assay, measured on both the DropSense16 and Spectramax (Molecular Devices). DropSense16 data (red) shows close resemblance with Spectramax (blue).

App Report

Four report types are generated: an HTML file, XML, txt and a CSV file (for import in MS Excel). Each report contains concentration, absorbance at $\lambda 1$ and $\lambda 2$ (blue circles), formula describing the standard curve, R^2 , type of curve and information. When 2 concentrations match 1 absorbance value, ambiguous will be displayed in the information field. If samples have an absorbance not matching the standard curve, out of range will be displayed with or without an estimated value, depending whether this absorbance could cross the curve. In addition, the HTML report contains the graphs of each separate measurement. These reports are easily exported from the DropSense16 to a USB key, network folder or to a smart device (using the QR code). An additional DropSense16 specific measurement file (.bin) is also exported.

Discussion

These colorimetric applications can be used to quantify protein samples in combination with the appropriate colorimetric assay. Assays can be performed as described by supplier.



Contact information

Trinean nv
Dulle Grietlaan 17/3
9050 Gentbrugge
Belgium

Tel: +32/9 272 75 35
Fax: +32/9 272 75 39
E-mail: info@trinean.com
www.trinean.com