Protein quantification on Infinite™ 200 with injectors

Modified Lowry protein assay

Introduction

Protein quantification is often required before proceeding with protein samples for isolation, chromatographic, or electrophoretic analysis, or for use in functional or immunohistochemical assays.

For general purpose protein quantification, two different groups of techniques are known; the protein-dye binding (Coomassie®) and the protein-copper chelation chemistry.

The modified Lowry protein assay is an enhanced copper chelation method, based on the original protocol of Oliver Lowry's method [1] but with increased convenience because of a more stable formulated product. In the first step of this assay, a tetradentate protein-copper complex is formed in alkaline solution, which reduces the Folin-Ciocalteu reagent added in the second step. The reaction product is blue colored and water-soluble and can be measured from 650 to 750 nm (see figure 1).

Like other protein assays, the modified Lowry protein assay requires a standard curve each time the protein concentration of a sample has to be determined. The protein concentration range of the standard curve with BSA lies between 1-1500 µg/ml.

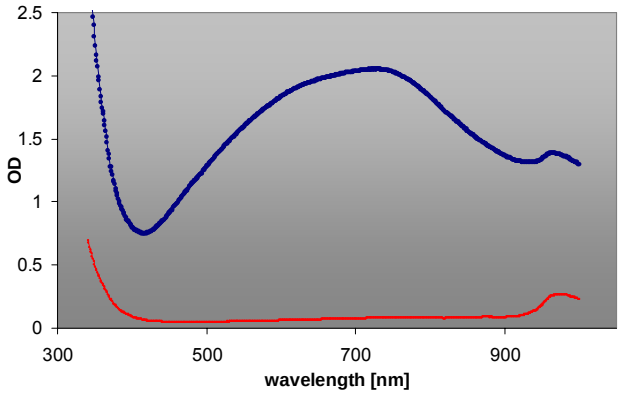

Figure 1: Absorbance scan from 300-1000 nm of the modified Lowry protein assay reaction with (—) and without (—) BSA measured on the Infinite M200 reader.

The modified Lowry protein assay can also be performed in microtiter plates, if small volumes of protein samples or a larger number of samples have to be measured.

For a precise determination of protein concentrations, the modified Lowry protein assay requires accurate pipetting with an exactly timed addition of the Folin-Ciocalteu reagent, mixing, incubation and reading. This limits the number of samples which can be processed if the assay is performed manually.

In this note, we show how Tecan´s Infinite injector system, in combination with the Infinite 200 instrument series, automatically performs the modified Lowry protein assay.

Using this system the injection of the two different reagents (modified Lowry and Folin-Ciocalteu reagent), mixing the reagents, incubation and measurement of the absorbance, can all be performed with only one instrument set-up.

Material and methods

Instruments

- Infinite F200 filter-based detection system with injector (Tecan, Austria)
- Infinite M200 Quad4 Monochromator™ detection system with injector system (Tecan, Austria)

Microplates

- 96-well flat transparent microplate (Corning, Germany)

Reagents and assay performance

Reagents

- Modified Lowry Protein Assay Kit (Pierce, Germany) containing the modified Lowry protein assay reagent, 2 N Folin-Ciocalteu reagent and bovine serum albumin (BSA) standard (2 mg/ml)
- water, ultrapure

Reagent preparation

Modified Lowry protein assay
The supplied 2N Folin-Ciocalteu reagent is diluted 1:1 with water and should be used within one day.

BSA dilution series for standard curve
BSA (2 mg/ml) was used for preparing the dilutions series for the standard curve (see table 3).

Vial	Volume H_2O (µl)	Volume BSA (µl)	BSA conc. (µg/ml)
A	250	750 of stock	1500
B	625	625 of stock	1000
C	310	310 of A	750
D	625	625 of B	500
E	625	625 of D	250
F	625	625 of E	125
G	800	200 of F	25
H	800	200 of G	5
I	800	200 of H	1
J	1000	0	0

Table 1: Preparation of the BSA standards for the modified Lowry protein assay.

Plate definition file

The working volume within a well in a flat 96-well microplate is generally 200 µl. For application of the modified Lowry protein assay the working volume has to be set to 260 µl in the plate definition file.

Plate definitions can be easily set with i-control™ software: in the menu bar, select *Settings* then *Plate definition*; choose a flat transparent 96-well plate and create a new name for this plate; then click on *Well geometry* and change the working volume to 260 µl and press *ok*.

Modified Lowry protein assay script

Create a script according to steps described below using the instruments i-control software.

Steps	Script for F and M200 with injector system	
1	**Plate definition**	[COS96ft]-Corning 96 flat transparent - **PLATE DEFINITION - Volume!**
2	**Part of plate**	Select rows
3	**Dispense**	Injector A, Refill for every dispense **200 µl** Speed 200 µl/sec, refill speed 100 µl/sec
4	**Shaking**	30 sec, amplitude 2 min, orbital
5	**Wait**	10 min
6	**Dispense**	Injector B **20 µl** Speed 200 µl/sec, refill mode standard
7	**Shaking**	30 sec, amplitude 1 mm
8	**Wait**	30 min
9	**Absorbance**	750 nm, number of reads 25
10	**Move plate**	out

Measurement settings

Infinite F200	
Parameter	Setting
Mode	Absorbance
Wavelength	750 nm
Bandwidth	10 nm
Number of reads	25

Infinite M200	
Parameter	Setting
Mode	Absorbance
Wavelength	750 nm
Bandwidth	9 nm
Number of reads	25

Table 2: Measurement settings for absorbance measurements on Infinite F200 and M200

Assay description

Before starting the measurement, the injector system has to be washed and primed with the reagents. Depending on the total volume needed for all assays, fill one flask of the injector with modified Lowry reagent and put it into the injector A. Fill a second flask with 1x Folin-Ciocalteu reagent and put the flask into injector B. Prime the two injectors as described in the instructions for use.

Pipette manually 40 µl of each dilution of the BSA standard curve and 40 µl of each sample to be analyzed into corresponding wells of the microplate.

Put the plate into the Infinite reader and start the script for performing the assay and absorbance measurement. The i-control software automatically exports the data obtained in Excel® for further analysis.

For optimal performance of the injectors it is recommended to wash the injectors after use as described in the instructions for use.

Results

The modified Lowry protein assay was performed using the Infinite F200 and the Infinite M200 instruments, both equipped with an injector system. BSA standards were pipetted in triplicate and the assay was performed using the script described above. The measured absorbance at 750 nm is directly proportional to the amount of protein. Both instruments, the monochromator-based Infinite M200 and the filter-based Infinite F200, show the same color response curves. As already described in the literature, and as can also be seen from the graphs, the color response curve is not linear.

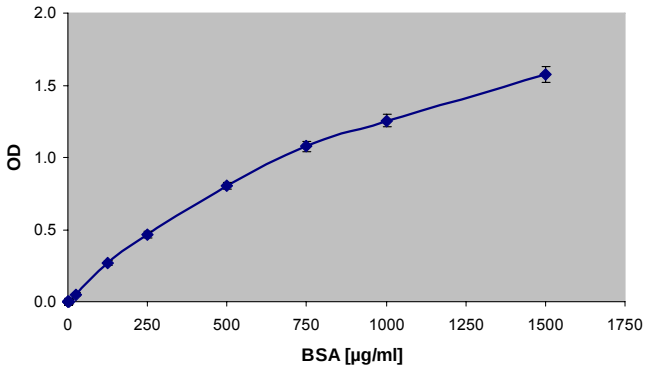

Figure 2: Absorbance measurements with the Infinite F200 of BSA from 0-1500 µg/ml with the modified Lowry protein assay.

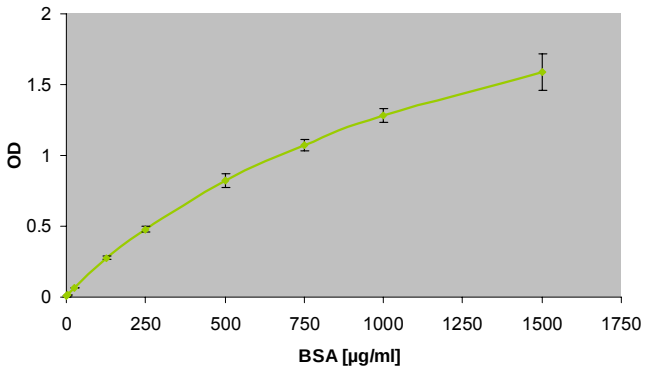

Figure 3: Absorbance measurements with the Infinite M200 of BSA from 0-1500 µg/ml with the modified Lowry protein assay.

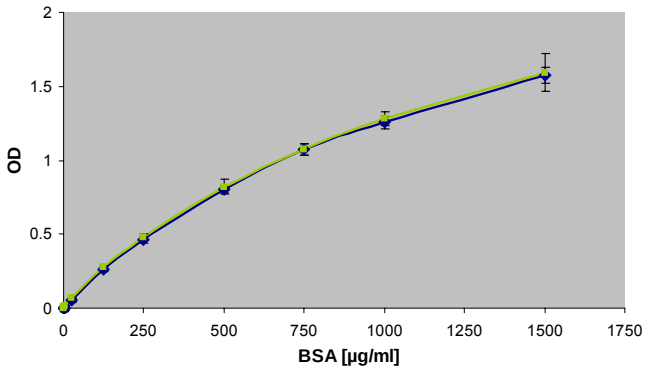

Figure 4: Comparison of the two protein-color response curves measured with the Infinite F200 and Infinite M200. The green curve indicates OD measured on the Infinite M200 while the blue curve displays OD values measured on the Infinite F200.

Discussion

There are various types of protein assays available, all of them with a different focus. Some are rapid and cheap, some declare insensitivity against interfering compounds and others highest sensitivity. The modified Lowry protein assay is based on Lowry's method, which is one of the most cited methods for protein quantification.

To obtain reproducible data, one has to keep precisely accurate time-dependent conditions, which becomes more and more cumbersome with a practical handling limit of about 20 samples manually. This challenge can be easily avoided when using an automated system like the Infinite 200 series of instruments equipped with an injector. Injecting, mixing, incubation and measuring are done within only one instrument set-up and up to 96 samples can be easily processed using the microplate format. All steps are easy to program with i-control software and, for further data analysis, calculation and presentation, Tecan´s Magellan™ software is the tool of choice.

Virtually any assay with one or two different reagents to be added to a sample can be performed using the injector system. Additionally, shaking and incubation times can be programmed and are performed automatically without the need for moving the plate manually from a shaker to an incubator and finally to the reader. This saves expensive working time and ensures an exactly followed workflow.

Conclusion

For protein assays in general, and especially for the modified Lowry protein assay, the reader-injector system is an ideal solution avoiding pipetting and workflow errors, and resulting in consistent and traceable data.

Tecan´s Infinite 200 instrument series extended with the injector system offers a multi-functional system, from injection of reagents, to mixing, incubation and measuring, with all steps performed within one instrument set-up.

List of abbreviations

BSA	Bovine serum albumin
OD	Optical density

Literature

[1] Lowry *et al*.: Protein Measurement with Folin Phenol Reagent. J Biol Chem, 193, 265 – 275, 1951

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395 179 V.1.0, 08-2007