LIBIOS

Dynamic Test Kits for R&D and Quality Control

Lowry Protein Assay Kit

KB-03-004

1200 test (96 well plate)

BQCkit

A brand of BioQuoChem

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All chemicals should be handled with care

- This kit is for R&D use only

Introduction

Lowry Protein Quantification Assay is based on Lowry method, first described in 1951. The method relies on two different reactions. The first is the formation of a copper ion complex with amide bonds, forming reduced copper in alkaline solutions. This is called a Biuret chromophore and is commonly stabilized by the addition of tartrate. The second reaction is reduction of Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate), primarily by the reduced copper-amide bond complex as well as by tyrosine, tryptophan, histidine, cystine, and cysteine residues in protein.The monovalent copper ion catalyzes the latter reaction.

The reduced Folin-Ciocalteu reagent is blue and thus detectable with a spectrophotometer in the range of 500 to 750 nm. The Biuret reaction itself is not very sensitive. Using the Folin-Ciocalteu reagent to detect reduced copper makes the Lowry assay nearly 100 times more sensitive than the Biuret reaction alone.

Materials

BQCkit Lowry Protein Quantification Assay kit KB-03-004 1200 tests contains:

Product	Quantity	Storage
Lowry Reagent A	2 bottles	RT
Lowry Reagent B	1 vial	RT
Lowry Reagent C	1 bottle	RT
Protein Standard*	5 vials	4ºC

*This reagent is stable during 10 days at Room Temperature and is shipped in these conditions. Once received is recommended to keep it at 4ºC.

Assay Principle

The reaction occurring in the Lowry method takes place in the following phases:

1. Pre-reaction of protein with alkaline Cu^{2+}, in presence of tartrate to avoid precipitation. It is essentially identical than Biuret reaction, forming a coordination complex between copper and peptide nitrogen.

2. Reduction of the Folin-Ciocalteu reagent by the reduced copper-amide bond complex as well as by tyrosine and tryptophan residues. The reduced Folin-Ciocalteu reagent presents a dark blue color, which is measured colorimetrically.

The colored complex, whose composition is unknown; it has two absorption maxima at wavelengths of 560 and 680 nm. The choice of either depends on the protein concentration of the sample.

The Lowry assay is relatively sensitive but requires more time than other assays and is susceptible to many interfering compounds. Table 1 shows compatible substance concentrations in BQC Lowry Protein Quantification Kit.

Assay Principle

Table 1. Reagents compatible with the kit protein assay when the standard procedure is used*

Substance	Compatible concentration
Salts/Buffers	
Ammonium sulphate	---
Asparagine	5 mM
Cesium bicarbonate	50 mM
Glycine	100 mM
HEPES, pH 7.5	1 mM
Imidazole	25 mM
MES, pH 6.1	125 mM
Sodium acetate, pH 4.8	200 mM
Sodium azide	0,2 %
Sodium bicarbonate	100 mM
Sodium chloride	1 mM
Sodium phosphate	100 mM
Tris	10 mM
Detergents	
Brij-35	0.031 %
Brij-56, Brij-58	0.062 %
CHAPS	0.062 %
CHAPSO	0.031 %
Lubrol PX	0.031 %
Octyl -glucoside	0.031 %
Nonidet P-40	0.016 %
SDS	1.0 %
SPAN 20	0.25 %
Triton® x-100, x-114, x305, x-405	0.031 %
Tween®-20	0.062 %
Tween®-80	0.031 %
Chelating agents	
EDTA	1 mM
EGTA	1 mM
Sodium citrate	100 mM

Assay Principle

Reducing and thiol-containing agents	
Ascorbic acid	1 mM
Cysteine	1 mM
Dithioerythritol (DTE)	
Dithiothreitol (DTT)	
Glucose	100 mM
Melibiose	25 mM
2-mercaptoethanol	1 mM
Potassium thiocyanate	100 mM
Thimerosal	0.01 %

Reagents and solvents	
Acetone	10 %
Acetonitrile	10 %
Aprotinin	10 mg/L
DMF	10 %
DMSO	10 %
Ethanol	10 %
Glycerol (Fresh)	10 %
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Methanol	10 %
Phenol Red	0.01 mg/mL
PMSF	1 mM
Sodium hydroxide	100 mM
Sucrose	7.5 %
TLCK	0.01 mg/L
TPCK	0.1 mg/L
Urea	3 M

*The amount listed is the maximum amount of the material allowed in the protein sample without causing a noticeable interference.

Note: This is not a complet compatibility chart. There are many substances that can affect different proteins in different ways. One may assay the protein of interest in deionized water alone, then in buffer with possible interfering substances. Comparison of the readings will indicate if interference exists.

Reagent Preparation

Lowry Assay Mix:

Mix Lowry Reagent B with Lowry Reagent A in a ratio 1:100.

Prepare fresh daily.

Lowry Working Solution:

Dilute Lowry Reagent C with an equal volume of ultrapure water to prepare the desired volume.

Prepare diluted reagent fresh daily.

Assay Protocol

There are two protocols to perform the assay. The micro assay is for samples with low protein concentrations. The 96 well plate assay is for those who wish to perform the Lowry assay in plate format. If another format is required, extrapolate the volume of samples and reagents.

BQC Lowry Protein Quantification Kit Micro assay (6.5 mL)

1. Pipette 1 mL of each standard or unknown sample solution into separate clean test tubes. Refer to the Table 2 as a guide for diluting the protein standard. For the diluents, use the same buffer as in the samples.
2. To each tube, add 5 mL of freshly prepared Lowry assay mix and thoroughly vortex.
3. Incubate tubes 10 min at room temperature.
4. Add 0.5 mL of Lowry Working Solution to each tube and vortex immediately.
5. Incubate 30 min at room temperature.
6. Vortex the tubes, zero the spectrophotometer with the blank, and measure the absorbance at 660 nm.

Table 2. Microassay Standard Dilutions

Sample	Standard [mL]	Diluent [mL]	Protein [mg]
S1 (Blank)	---	2	---
S2	0.025	1.975	0.125
S3	0.05	1.95	0.25
S4	0.1	1.9	0.5
S5	0.2	1.8	1

Note: The absorbance will not change significantly if all the samples are read at the same time. The Lowry assay is not an end point assay, so samples will change in absorbance if too much time elapses between sample readings. The typical time that elapses during the reading of samples (‹10 min) does not usually result significant changes in absorbance.

BQC Lowry Protein Quantification Kit Microplate

1. Pipette 40 µL of each standard and unknown sample replicate into a microplate well. Refer to the Table 3 as a guide for diluting the protein standard. For the diluents, use the same buffer as in the samples.
2. Add 200 µL of freshly prepared Lowry Assay Mix and immediately mix the microplate for 30 seconds.
3. Incubate microplate exactly 10 min at room temperature.
4. Add 20 µL of freshly prepared Lowry Working Solution to each well and immediately mix the microplate for 30 seconds.
5. Incubate 30 min at room temperature. Measure the absorbance at 660 nm on a plate reader.

Table 3. Microplate Standard Dilutions

Sample	Standard [µL]	Diluent [µL]	Protein [µg]
S1 (Blank)	---	200	---
S2	2.5	197.5	5
S3	5	195	10
S4	10	190	20
S5	20	180	40

Assay Protocol

Note: The absorbance will not change significantly if all the samples are read at the same time. The Lowry assay is not an end point assay, so samples will change in absorbance if too much time elapses between sample readings. The typical time that elapses during the reading of samples (‹10 min) does not usually result significant changes in absorbance.

Plate set up

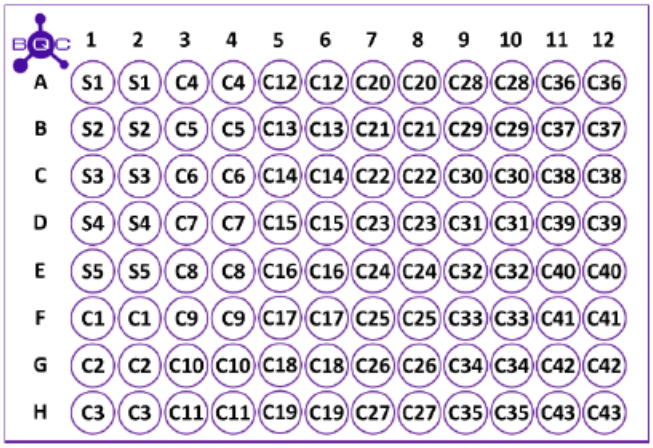

Figure 1. 96-well plate filling format

S1-S5 = Standards
C1-C43 = Samples

Attention

- This scheme is just a recommendation of how to perform the assay.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user´s discretion to do so.

Data Analysis

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.
2. Create a standard curve by plotting A660 nm (y-axis) vs BSA standard (µg) (x-axis). Determine the unknown sample concentration using the standard curve.
3. The level of detection of the assay is lower for the microplate assay when compared with the microassay due to a shorter light path used in the microplate reader.
4. Standard curve example for microplate assay procedure is shown in Figure 2.

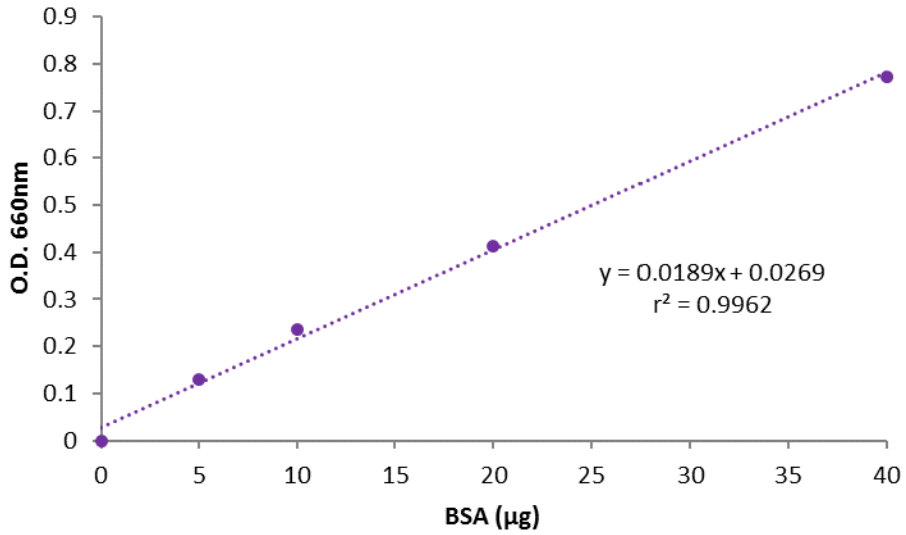

Figure 2. Typical standard curve for Lowry assay

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Expiration date: 1 year from the date of delivery

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