INSTRUCTIONS

Modified Lowry Protein Assay Kit

23240

0389.6

Number	Description
23240	**Modified Lowry Protein Assay Kit**, sufficient reagents for 480 test tubes or 2400 microplate assays
	Kit Contents:
	Modified Lowry Protein Assay Reagent, 480mL, containing cupric sulfate, potassium iodide, and sodium tartrate in an alkaline sodium carbonate buffer
	2N Folin-Ciocalteu Reagent, 50mL
	Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules containing bovine serum albumin (BSA) at a concentration of 2.0mg/mL in 0.9% saline and 0.05% sodium azide; store at 4°C or room temperature
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature in two separate packages.

IMPORTANT NOTE: To comply with Department of Transportation (DOT) shipping regulations, the 2N Folin-Ciocalteu Reagent is shipped in a separate package from the remaining components. Upon receipt of both packages, components may be placed together in a single kit box for storage.

Table of Contents

Introduction

For many years, Lowry's method was the most widely used and cited procedure for protein quantitation. The procedure involves reaction of protein with cupric sulfate and tartrate in an alkaline solution, resulting in formation of tetradentate copper-protein complexes. When the Folin-Ciocalteu Reagent is added, it is effectively reduced in proportion to these chelated copper complexes, producing a water-soluble product whose blue color can be measured at 750nm. For the original Lowry method, the alkaline copper-tartrate reagent (Reagent C) must be prepared fresh daily from two other reagents (Reagents A and B). Pierce has developed a modified cupric sulfate-tartrate reagent that replaces individual Reagents A and B of the original Lowry method with a single stable reagent that substitutes for Reagent C. The color response curves for the Modified Lowry Protein Assay and the original Lowry method have nearly 100% correlation. Accordingly, the Thermo Scientific Modified Lowry Protein Assay Kit is ideal for loyal Lowry method users who would like the increased convenience of a stable, pre-formulated product.

As with other protein assay procedures, the Modified Lowry Protein Assay produces slightly different color response curves for different proteins and can be affected by certain components in the sample buffer. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA), which is included in this kit. A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard (see Related Thermo Scientific Products) may be used when assaying immunoglobulin samples.

Preparation of Standards and Folin-Ciocalteu Reagent

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as your sample. The pooled contents of two ampules of 2.0mg/mL Albumin Standard is sufficient to prepare a set of diluted standards for the working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard. When using the Microplate Procedure, it is sufficient to use one ampule of Albumin Standard and prepare half as much volume of each standard dilution (e.g., for vial A, add 125µL diluent to 375µL of BSA Stock).

Table 1. Preparation of Diluted Albumin (BSA) Standards

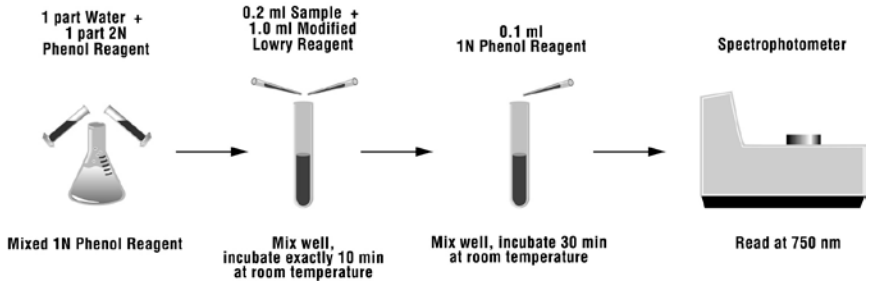
Dilution Scheme for Test Tube and Microplate Procedure (Working Range = 1-1500µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	250µL	750µL of Stock	1500µg/mL
B	625µL	625µL of Stock	1000µg/mL
C	310µL	310µL of vial A dilution	750µg/mL
D	625µL	625µL of vial B dilution	500µg/mL
E	625µL	625µL of vial D dilution	250µg/mL
F	625µL	625µL of vial E dilution	125µg/mL
G	800µL	200µL of vial F dilution	25µg/mL
H	800µL	200µL of vial G dilution	5µg/mL
I	800µL	200µL of vial H dilution	1µg/mL
J	1000µL	0	0 µg/mL = Blank

B. Preparation of 1X Folin-Ciocalteu Reagent

Prepare 1X (1N) Folin-Ciocalteu Reagent by diluting the supplied 2X (2N) reagent 1:1 with ultrapure water. Because the diluted reagent is unstable, prepare 1X Folin-Ciocalteu Reagent on the same day of use. Each test replicate requires 100µL of 1X Folin-Ciocalteu Reagent in the Test Tube Protocol and 20µL of 1X Folin-Ciocalteu Reagent in the Microplate Protocol.

Procedure Summary (Test Tube Procedure):



Test Tube Procedure

1. Pipette 0.2mL of each standard and unknown sample replicate into an appropriately labeled test tube.
2. At 15-second intervals, add 1.0mL of Modified Lowry Reagent to each test tube. Mix well and incubate each tube at room temperature (RT) for exactly 10 minutes.
3. Exactly at the end of each tube's 10-minute incubation period, add 100µL of prepared 1X Folin-Ciocalteu Reagent, immediately vortex to mix the contents. Maintain the 15-second interval between tubes established in Step 2.
4. Cover and incubate all tubes at RT for 30 minutes.
5. With the spectrophotometer set to 750nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
6. Subtract the average 750nm absorbance values of the Blank standard replicates from the 750nm absorbance values of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 750nm value for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure

1. Pipette 40µL of each standard and unknown sample replicate into a microplate well (Product No. 15041).
2. Add 200µL of Modified Lowry Reagent to each well at nearly the same moment using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.
3. Cover (e.g., Sealing Tape for 96-Well Plates, Product No.15036) and incubate microplate at room temperature (RT) for exactly 10 minutes.
4. Add 20µL of prepared 1X Folin-Ciocalteu Reagent to each well using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.
5. Cover and incubate microplate at RT for 30 minutes.
6. Measure the absorbance at or near 750nm on a plate reader.
7. Subtract the average 750nm absorbance value of the Blank standard replicates from the 750nm value of all other individual standard and unknown sample replicates.
8. Prepare a standard curve by plotting the average Blank-corrected 750nm values for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contained a chelating agent (e.g., EDTA, EGTA)	Dialyze, desalt, or dilute sample, or remove interfering substances from sample using Product No. 23215
Blank 562nm absorbance value is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, altered working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 750nm
A precipitate forms upon addition of reagent to samples	Sample contained a surfactant (detergent)	Dialyze or desalt sample, or remove interfering substances from sample using Product No. 23215
	Sample contained potassium ions	
All tubes (including blank) are dark purple	Sample contained a reducing agent	Dialyze or dilute sample, or remove interfering substances from sample using Product No. 23215
	Sample contained a thiol	
Need to measure color at a different wavelength	Spectrophotometer or plate reader did not have 750nm filter	Color may be measured at any wavelength between 650nm and 750nm, although the slope of the standard curve and overall assay sensitivity will be reduced

A. Interfering substances

Certain substances are known to interfere with the Modified Lowry Protein Assay including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, minimize the following substances as components of the sample buffer:

Catecholamines and Uric Acid	Impure Glycerol	Impure Sucrose
Cysteine	Hydrogen Peroxide	Thiols, disulfides
Detergents (cause precipitation)	Hydrazides	Tris, Tricine , Potassium ions
Copper chelators (e.g, EDTA, EGTA)	Lipids	Tryptophan, Tyrosine

Maximum compatible concentrations for many substances in the Test Tube Procedure are listed in Table 2 (see last page of these instructions). Substances were compatible at the indicated concentration in the Test Tube Procedure if the error in protein concentration estimation caused by the presence of the substance in the sample was less than or equal to 10%. Blank-corrected 750nm absorbance values (for a 1000µg/mL BSA standard + substance) were compared to the net 750nm values of the same standard prepared in 0.9% saline.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Modifed Lowry Protein Assay may be overcome by one of several methods.

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes.
- Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in the Modified Lowry Protein Assay Reagent. Alternatively, Product No. 23215 may be used (see Related Thermo Scientific Products).

  Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

Related Thermo Scientific Products

15041	**Pierce 96-Well Plates,** 100/pkg.
15075	**Reagent Reservoirs,** 200/pkg.
15036	**Sealing Tape for 96-Well Plates,** 100/pkg.
23208	**Bovine Serum Albumin Standard Pre-Diluted Set**, 7 × 3.5mL
23212	**Bovine Gamma Globulin Standard Ampules, 2mg/mL,** 10 × 1mL
23213	**Bovine Gamma Globulin Standard Pre-Diluted Set,** 7 × 3.5mL
23227	**Pierce BCA Protein Assay Kit**
23236	**Coomassie Plus Protein Assay Kit**
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**

Additional Information

A. Please visit the Thermo Scientific web site for additional information on this product.

B. Response characteristics for different proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins. These differences relate to amino acid sequence, pI, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods utilize BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined (Figure 1). However, if great accuracy is required, the standard curve should be prepared from a pure sample of the target protein to be measured.

Table 3 shows typical protein-to-protein variation in color response with the Modified Lowry Protein Assay. All proteins were tested at a concentration of 1000µg/mL using the Test Tube Procedure. The average net color response for BSA was normalized to 1.00 and the average net color response of the other proteins is expressed as a ratio to the response of BSA.

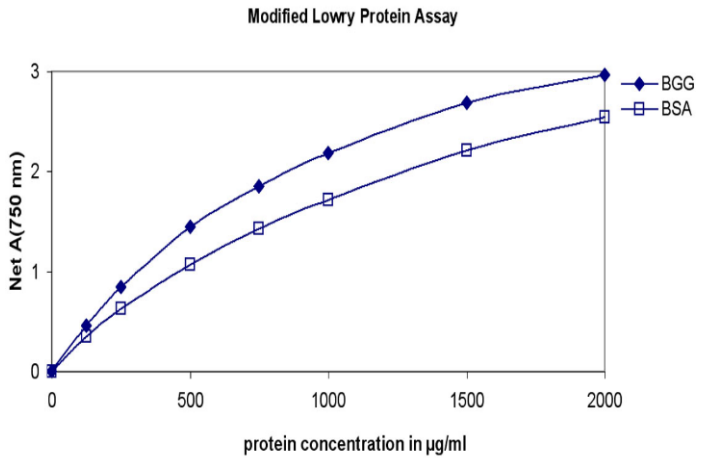


Figure 1: Typical color response curves for BSA and BGG using the Test Tube Protocol.

Table 3. Protein-to-Protein Variation 750nm absorbance ratios for proteins relative to BSA using the Test Tube Procedure.

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)	
Protein Tested	**Ratio**
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.94
α-Chymotrypsinogen, bovine	1.17
Cytochrome C, horse heart	0.94
Gamma globulin, bovine	1.14
IgG, bovine	1.29
IgG, human	1.13
IgG, mouse	1.20
IgG, rabbit	1.19
IgG, sheep	1.28
Insulin, bovine pancreas	1.12
Myoglobin, horse heart	0.90
Ovalbumin	1.02
Transferrin, human	0.92
	1.09
Standard Deviation	**0.13**
Coefficient of Variation	**11.9%**

C. Alternative Total Protein Assay Reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Thermo Scientific Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236), which is less sensitive to such substances. If incompatibilities with detergents cannot be overcome, try the BCA Protein Assay Kit (Product No. 23227).

D. Cleaning and Re-using Glassware

Exercise care when re-using glassware. Glassware must be cleaned and given a thorough final rinse with ultrapure water.

General References

Bensadoun, A. and Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. *Anal Biochem* **70**:241-50.

Davies, E.M. (1988). Protein assays: A review of common techniques. *Am Biotech Lab* 28-37.

Legler, G., *et al.* (1985). On the chemical basis of the Lowry protein determination. *Anal Biochem* **150**:278-87.

Lowry, O.H., *et al.* (1951). Protein measurement with the Folin Phenol Reagent. *J Biol Chem* **193**:267-75.

Ohnishi, S.T. and Barr, J.K. (1978). A simplified method of quantitating protein using the biuret and phenol reagents. *Anal Biochem* **86**:193-200.

Vallejo, C.G. and Lagunas, R. (1970). Interferences by sulfhydryl, disulfide reagents, and potassium ions on protein determination by Lowry's method. *Anal Biochem* **36**:207-12.

Table 2. Compatible Substance Concentrations in the Modified Lowry Protein Assay.

Substance	Compatible Concentration
Salts/Buffers	
Ammonium sulfate	--------
Asparagine	5mM
Cesium bicarbonate	50mM
Glycine	100mM
HEPES, pH 7.5	1mM
Imidazole, pH 7.0	25mM
MES, pH 6.1	125mM
Sodium acetate, pH 4.8	200mM
Sodium azide	0.2%
Sodium bicarbonate	100mM
Sodium chloride	1M
Sodium phosphate	100mM
Tris	10mM
Detergents	
Brij®-35	0.031%
Brij-56, Brij-58	0.062%
CHAPS	0.062%
CHAPSO	0.031%
Lubrol® PX	0.031%
Octyl β-glucoside	0.031%
Nonidet P-40 (NP-40)	0.016%
SDS	1.0%
Span® 20	0.25%
Triton® X-100, X-114, X-305, X-405	0.031%
Tween®-20	0.062%
Tween-80	0.031%

A dashed-line entry indicates that the material is incompatible with the assay.

Substance	Compatible Concentration
Chelating agents	
EDTA	1mM
EGTA	1mM
Sodium citrate	100mM
Reducing & Thiol-Containing Agents	
Ascorbic acid	1mM
Cysteine	1mM
Dithioerythritol (DTE)	--------
Dithiothreitol (DTT)	--------
Glucose	100mM
Melibiose	25mM
2-Mercaptoethanol	1mM
Potassium thiocyanate	100mM
Thimerosal	0.01%
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10mg/L
DMF	10%
DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrochloric Acid	100mM
Leupeptin	10mg/L
Methanol	10%
Phenol Red	0.01mg/mL
PMSF	1mM
Sodium Hydroxide	100mM
Sucrose	7.5%
TLCK	0.01mg/L
TPCK	0.1mg/L
Urea	3M

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