INSTRUCTIONS

Modified Lowry Protein Assay Reagent Kit

23240

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0389w

Number	Description
23240	**Modified Lowry Protein Assay Reagent Kit**, sufficient reagents for 480 test tube or 2,400 microplate assays

Kit Contents:

Modified Lowry Protein Assay Reagent, 480 ml, containing cupric sulfate, potassium iodide, and sodium tartrate in an alkaline sodium carbonate buffer.

2N Folin-Ciocalteu Reagent, 50 ml

Albumin Standard Ampules, 2 mg/ml, 10 x 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide

Storage: Upon arrival store at 4°C. Product shipped at ambient temperature.

Note: Discard any kit reagent that shows discoloration or evidence of microbial contamination.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

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Introduction

For many years, Lowry's[4] 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 750 nm. 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 Modified Lowry Protein Assay Reagent 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 Pierce 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 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.

Note: 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 75 µl diluent to 375 µl of BSA Stock).

Table 1: Preparation of Diluted Albumin (BSA) Standards

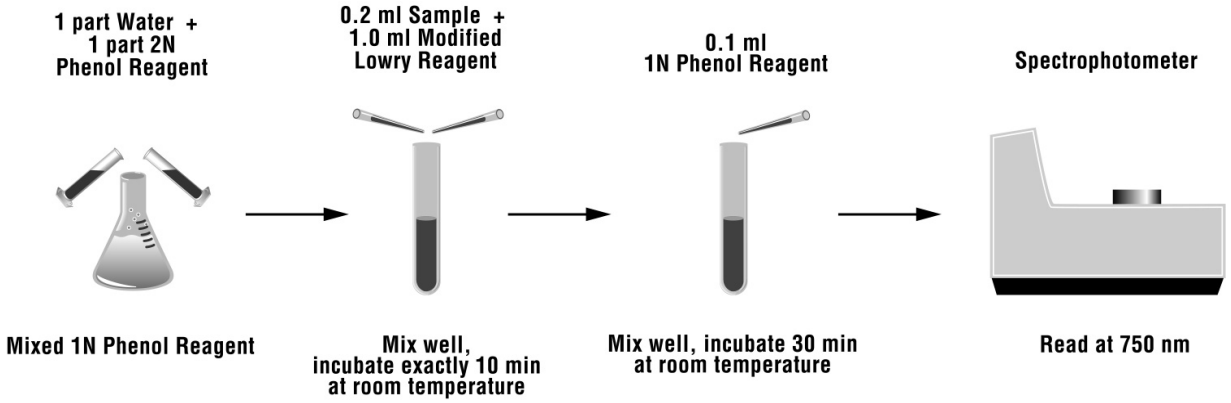
Dilution Scheme for Test Tube and Microplate Procedure (Working Range = 1–1,500 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	150 µl	750 µl of Stock	1,500 µg/ml
B	625 µl	625 µl of Stock	1,000 µg/ml
C	310 µl	310 µl of vial A dilution	750 µg/ml
D	625 µl	625 µl of vial B dilution	500 µg/ml
E	625 µl	625 µl of vial D dilution	250 µg/ml
F	625 µl	625 µl of vial E dilution	125 µg/ml
G	800 µl	200 µl of vial F dilution	25 µg/ml
H	800 µl	200 µl of vial G dilution	5 µg/ml
I	800 µl	200 µl of vial H dilution	1 µg/ml
J	1000 µl	0	0 µg/ml = Blank

B. Preparation of 1X Folin-Ciocalteu Reagent

Prepare 1X (1 N) Folin-Ciocalteu Reagent by diluting the supplied 2X (2 N) reagent 1:1 with ultrapure water. Because the diluted reagent is unstable, prepare only as much 1X Folin-Ciocalteu Reagent as will be used in one day. Each test replicate requires 100 µl of 1X Folin-Ciocalteu Reagent in the Test Tube Protocol and 20 µl of 1X Folin-Ciocalteu Reagent in the Microplate Protocol.

Procedure Summary (Test Tube Procedure)



Test Tube Procedure

1. Pipette 0.2 ml of each standard and unknown sample replicate into an appropriately labeled test tube.
2. At 15-second intervals, add 1.0 ml 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 100 µl 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 750 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
6. Subtract the average 750 nm absorbance values of the Blank standard replicates from the 750 nm absorbance values of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 750 nm 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 40 µl of each standard and unknown sample replicate into a microplate well.
2. Add 200 µl 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 and incubate microplate at room temperature (RT) for exactly 10 minutes.
4. Add 20 µl 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 750 nm on a plate reader.
7. Subtract the average 750 nm absorbance value of the Blank standard replicates from the 750 nm value of all other individual standard and unknown sample replicates.
8. Prepare a standard curve by plotting the average Blank-corrected 750 nm values for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.
9. **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 contains a chelating agent (e.g., EDTA, EGTA)	Dialyze, desalt, or dilute sample, or remove interfering substances from sample using Product No. 23215
Blank 562 nm absorbance value is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 750 nm
A precipitate forms upon addition of reagent to samples	Sample contains a surfactant (detergent)	Dialyze or desalt sample, or remove interfering substances from sample using Product No. 23215
	Sample contains potassium ions	

Continued next page

Troubleshooting, continued

All tubes (including blank) are dark purple	Sample contains a reducing agent	Dialyze or dilute sample, or remove interfering substances from sample using Product No. 23215
	Sample contains a thiol	
Need to measure color at a different wavelength	Colorimeter does not have 750 nm filter	Color may be measured at any wavelength between 650 nm and 750 nm, although the slope of 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 chelating reagents (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 750 nm absorbance values (for a 1,000 µg/ml BSA standard + substance) were compared to the net 750 nm 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 Pierce Products below).

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

Related Pierce Products

Number	Description
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin Fraction V (BSA) Set**, 7 x 3.5 ml of dilutions in the range of 125-2,000 µg/ml
23212	**Bovine Gamma Globulin Standard Ampules**, 2 mg/ml, 10 x 1 ml
23213	**Pre-Diluted Protein Assay Standards, Bovine Gamma Globulin Fraction II (BGG) Set**, 7 x 3.5 ml of dilutions in the range of 125-2,000 µg/ml
23227	**BCA Protein Assay Reagent Kit**, working range of 20-2,000 µg/ml
23236	**Coomassie® Plus Protein Assay Reagent Kit**, working range of 1-1,500 µg/ml
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**, sufficient reagents to pre-treat 500 samples to remove interfering substances prior to total protein quantitation

Additional Information

A. Please visit the Pierce 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 1,000 µg/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.

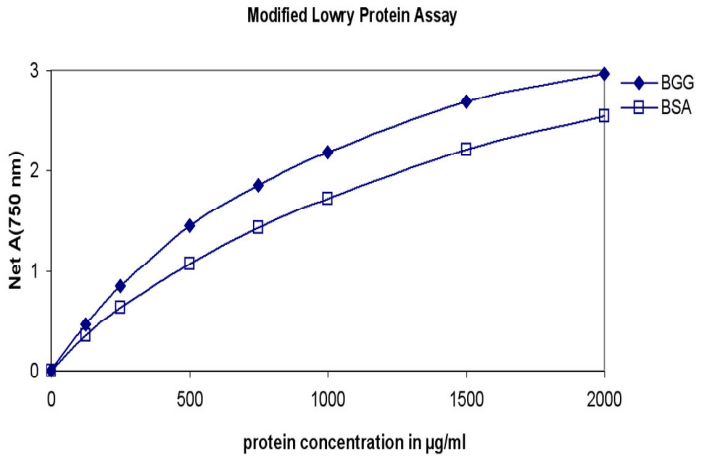


Table 3: Protein-to-Protein Variation

750 nm 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
Average ratio	**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 Coomassie® Plus Protein Assay Reagent Kit (Product No. 23236), which is less sensitive to such substances. If incompatibilities with detergents cannot be overcome, try BCA Protein Assay Reagent 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.

References

1. Bensadoun, A. and Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. *Anal. Biochem*. **70**, 241-250.
2. Davies, E.M. (1988). Protein assays: A review of common techniques. *Am. Biotech. Lab*. 28-37.
3. Legler, G., Muller-Platz, C.M., Mentges-Hettkamp, M., Pflieger, G. and Julich, E. (1985). On the chemical basis of the Lowry protein determination. *Anal. Biochem.* **150**, 278-287.
4. Lowry, O.H., Rosebrough, N.J., Farr, L. and Randall, R.J. (1951). Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **193**, 267-275
5. 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.
6. 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**, 207-212.

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

Substance	Compatible Concentration
Salts/Buffers	
Ammonium sulfate	--------
Asparagine	5 mM
Cesium bicarbonate	50 mM
Glycine	100 mM
HEPES, pH 7.5	1 mM
Imidazole, pH 7.0	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 M
Sodium phosphate	100 mM
Tris	250 mM
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	1 mM
EGTA	1 mM
Sodium citrate	100 mM
Reducing & 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%
Misc. Reagents & 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