IBIDD

Biopharmaceutical Development Program

Standard Operating Procedure

Title: Pierce Modified Lowry Protein Assay

SOP Number: 22146 **Revision Number: 01**

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Originator/Date: [redacted]

Approval/Date:

Approval/Date:

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1.0 Purpose

This procedure describes how to determine the amount of total protein in a sample using the Pierce Modified Lowry Protein Assay Kit. The Lowry method for determining protein concentration is essentially a biuret reaction. which incorporates the use of Folin-Ciocalteu reagent for enhanced color development. The procedure described below is the original Lowry protocol (see References) except that Reagent C (an alkaline copper solution that is a mixture of Reagent B and A) is replaced by Pierce's Modified Lowry Protein Assay Reagent.

2.0 Scope

This 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 al 750 nrn. The color response curves for the Modified Lowry Protain Assay and the original Lowry method have nearly 100% correlation. This procedure applies to Process Analytics staff who use this assay.

3.0 Authority and Responsibility

3.1 The Director. Process Analytics (PA) has the authority to define this procedure.

3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BOA).

3.3 PA personnel are responsible tor the performance of this procedure

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment.

4.·1 Modified Lowry Prolein Assay Reagent I<it (BDP PN 30283) containing:

Modified l.ow1·y Protein Assay Reagent (Pierce No. ·1856006);
2 N Folin-Ciocalteu Reagent (Pierce No. 1856007); and
Bovine Serum Albumin (BSA) Standard (BOP PN 30060); BGG No. 30059 or equivalent reagents, which may be ordered separately.

4.2 Saline, phosphate buffered saline or other appropriate diluent (i.e., formulation buffer of test samples).

4.3 88ckman DU650i Sp8d rophotometer, or equivalent.

4.4 Disposablepolystyrene cuvettes: 1.5 ml semi-micro (BDP PN 20070), 4.5 mL standard (Fisher **PN** 14-385-942), or BOP approved equivalent cuvettes.

4.5 Water, HPLC grade or better.

4.6 Assorted Micro-pipets, tips, culture tubes and laboratory glassware.

5.0 Procedure

5 1 Prepa ra tion of Solutions and Standards

5 1 1 Record the lot number of the Modified Lowry Protein Reagent, and its expiration date on the Lowry Protein Assay D:::ita Sheet (Attachment 1).

5.1.2 Prepare 1X {1N) Folin-Ciocalteu reagent by diluting the supplied 2X reagent (2N) with and equal volume of water. Prepare fresh reagent each day as it is unstable in the diluted form. Record the lot number of the Folin-Ciocalteu reagent on the Lowry Protein Assay Data She

5.1.3 Record the concentration and lot number of the BSA Standard on the Lowry Protein Assay Data Sheet.

5.1.4 Prepare a set of protein standards of known concentration by diluting the BSA standard solution provided with this kit, or any other suitable standard, in the same diluent as Ihe unknown samples. The set of standards should consist of eight dilutions of BSA over Ihe range of1-1500 µg/ml and should cover the range of protein concentrations being used. Record the dilutiondetails on the LowryProtein Assay Data Sheet. Triplicate standard samples require at least 600 **µL** of each concentration.

5 1.5 Also, analyze a sample of diluent buffer and use it as a background blank to zero the spectrophotometer. Record the description, including the lot number of the diluent buffer, on the Lowry Protein Assay Data Sheet (Attachment 1).

5.2 Sample Prepar ticm

5.2.1 Thi sam ple s and the protein standards must be diluted in thfl same diluent buffer.

5.2.2 Dilute the samples so that the approximate concentration is in the range of 20 - 1000 pg/ml or make a series of two -fold dilutions so that two or more of the dilutions will fall in this range. For samples of unknown protein concentration, the assay may require a second set of dilutions when all the readings in the firsl sel are out-of-rangefrom the standard curve.

5.2.3 Assay triplicates require at least 600 µL of eacti concentration.

5.3 3 Assay Protocol

5.3.1 Assay triplicate preparations of each concentration of standard and unknown sample.

5.3.2 To 200 }tl of each standard or unknown samples, add 1.0 mL of the Modified Lowry Protein Assay Reagent.

5.3.3 Vortex the samples.

5.3.4 Incubate the samples at room temperature (18-25°C) for 10 minutes.

5.3.5 Add 10_0 uL of the 1 N Folin-Ciocalteu Reagent while vortexing.

5.3.6 Incubate the samples at room temperature (18-25°C) for 30 minutes.

5.3.7 Measure the absorbance of the standards and samples at 750 nrn. Refer lo **SOP 22158, *Operation of the Beckman DU Series* 600 *Spectrophotometer*** for operation of the Beckman DU Series 600 spectrophotometerand to the Beckman DU Series 600 Spectrophotometer Protein Analysis Operating Instructions (Manual 517303B) for specific instructions for the protein assay.

This procedure Is made available lhrcugh federal funds from the Noti0t1ol Concer !stiM e. NIH, under cootract [redacted]

5.3.8 Prepare a standard curve by plotting the net (blank corrected) ahsorbance at 750 nm versus the protein concentration. Using this curve, determine the protein concentration of the unknown samples. The Beckman DU650i Spectrophotometer will perform this operation automatically when the PROTEIN operation is performed.

5.3.9 When using the PROTEIN operation on the Beckman DU650i Spectrophotometer, obtain printouts of the following data:

Protein Analysis Standards Window with A_{750} values;
Standard Curve of Concentration versus A_{700} values; and
Protein Analysis Samples Window with A_{700} values.

5.3.10 Alternatively, the raw A_{750}nm data may be analyzed using approved spreadsheet software such as Origin. SAS or Microsoft Excel.

5.3.11 When using second-party software for data analysis, printouts containing the same graphic data plots as in 5.3.9 are required.

Interpretation of Results

6.1 The protein concentration of the sample is obtained by comparison of the A15'j value of the sample with the standard curve. Solving for unknown's concentration "x."

fx = (A100- 'STD Y INll .:.RCEPT'/STD.SLOPE]

6.2 2 Since the Folin-Ciocalteu reagent reacts with tyrosine and tryptophanresidues, the Lowryassay is dependent on the unknown and the standard protein having a similar content of tyrosine + tryptophan/µg protein. This method is **NOT** appropriat e for prot eins with out tyrosine and tryptophan residues. or proteins known to contain significant proportions of tyrosine and trypotophan residues.

6.: Acceptance/Rejection Criteria

Standard Curve -.. If the P ROTEIN operation on the Beckman DU650 is used, after all the designated standards are analyzed, the standard curve can be displayed. Curve fit coefficients are displayed above the standard curve. Clicking on <ViewStats> from the Standards window will display the Standard Stats window which can be used to determine which, if any, of the standards could be rerun or deleted to improve the accuracy of the standard curve.

No more than 4 individual standard readings may be deleted (out of 24 total}.

Samples - If the value(s) obtained for an unknown sample is outside the range of the standard curve, repeat the assay with an appropriate set of dilutions to obtain values within the correct range.

6.4 The resulting standard curve R^2 valve must be 2: 0.90 for the assay to be valid.

This procedure is made available through federal fi:nds from the National Cancer Instilute. NIH, under contract [redacted]

7.0 Documentation

7.1 Following the run, the data should be available:

Protein Analysis Standards A_{750} Values;
Standard Curve; and
Protein Analysis Samples A_{750} Values.

7.2 Prepare a final report that shall include for each test article the printout of the A_{750} values of the standards, the standard curve and associated graphs, the A_{750} values of the test samples and the calculated protein concentrations of the test samples.

7.3 BQA Documentation will maintain the raw data, final report, documentation and protocols.

8.0 References and Related Documents

8.1 **SOP 22158** *Operation of the Beckman DU Series 600 Spectrophotometer*

8.2 Lowry, O.H., et al, J. Biol. Chem. 193, 267 - 275(1951).ist all other non BOP references.

8.3 Pierce Modified Lowry Protein Assay Reagent Kit Instructions (Manual 0389}.

8.4 Beckman DU Series 600 Spectrophotometer Operatin Instructions (Manual 5173008).

8.5 Beckman DU Series 600 Spectrophotometer Protein Analysis Operating Instructions {Manual 5173038).

9.0 Attachments

9.1 **Attachment 1** Form 22146-01, Process Analytics Laboratory Lowry Protein Assay Datasheet

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted]

Attachment 1

NCI•Fredenct
Fam .c.: 22146-01
501' No.: 21146
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Process AflaMics

Laboratory Lowiy Protein Assay Oatasheet
For Sample$ Analyzod with the Pierce Modliled Lowry Protein Msay

Da_u:. _ _ _ _ _ _ _ _ _ _ _ _ _ _ Keque 51ed By________________________

Number:____________________ Storo.g_e:._ _ _ _ _ _ _ _

Descpri tion _

Pierce MOdltled LOWIV l'rotem AA •Y Ktt K&')genlS (BOP PN 302!I;I:

M:Y.!Wia :I Lowry Proloin Reauenl BOPLoi Nu.._ _ _ _ _ _ _ _ _ .Exp__....
2 N Folin-Coo 1teu Reugen BDP Lui No. ....Exl__________________
St ood a d C ompoval: _ _ _ _ _ _ _ _ 8 0 P I , t Ne. Exll_ _ _ _ _ _ _ _

onuept Buffer for Samples and Sta n dards :

0"11Cri tio:1·_ _

Standards Solutions:

Na::;mc:.;e	..;St:::d;'(l'd_ I:;	......::0b:::de"n.:,t.,(u+LI	Cunccntralk> (IU.Jlml)

Comments and Inte,-p,etation:____

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Co :nJ)le edB / Da te_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

He 'l e-ma By/Oate:____

Tporcsm:1de avai lable :h f'OU9h federal funds from the National Cancer Institute. NIH. under conlrac t [redacted]