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1.0 Purpose

This procedure describes the BCA method for determining protein concentration.

2.0 Scope

This SOP refers to the use of the BCA protein detection method in order to detect and quantitate the amount of protein present in solution. This SOP is designed to ensure that putatively pure DNA/RNA in solution does not contain detectable levels of protein and to quantitate the protein concentration of proteinaceous solutions. The lower limit of linear response for this method is 1 μg/mL protein. A protein-spiked test sample is used to validate that the assay method has worked with the test sample since many test samples will result in O.D. 562 nm values equivalent to or slightly lower than the blank (no protein) standard sample. For further information regarding the methods and reagents in this SOP, refer to the Pierce BCA instruction manual or the Pierce web site: "www.piercenet.com."

3.0 Authority and Responsibility

3.1 The Director, Biopharmaceutical Quality Control (BQC) has the authority to define this procedure. BQA is responsible for the implementation of this procedure.

3.2 The Director of BQC is responsible for assignment of this procedure.

3.3 BQC personnel are responsible for the performance of this procedure

3.4 BQC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA)

3.5 BQC is responsible for reviewing the data and documentation of the results of this procedure.

3.6 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this operation.

4.0 Abbreviations

mg = milligrams

N = normal

BCA = Bicinchoninic Acid

μg = micrograms

mm = millimeters

mL = milliliters

nm = nanometers

μL = microliters

ND = not detected

5.0 Reagents

5.1 BCA Protein Assay Reagent (BCA Protein Assay Reagent Kit, BDP PN 30156)

5.1.1 Reagent A (MA): 1000 mL of Sodium Carbonate, Sodium Tartrate, and Bicinchoninic Acid in 0.1M NaOH

5.1.2 Reagent B (MC): 12 mL of 4.0% Cupric Sulfate Pentahydrate in water. Reagent B should be a blue-green color

5.1.3 Albumin (BSA) standard: 10 x 1 mL ampules containing BSA at a concentration of 2.0 mg/mL in a 0.9% saline and 0.05% sodium azide buffer. The standards are also available from Pierce separately as part number 23209. The extinction coefficient for BSA is 7.16 at A_{280} nm for a 10 mg/mL solution

5.1.4 Stock reagents (A, B) are stable for 1 year at room temperature from time of opening. Reagents A or B that have precipitated can be resuspended by warming to 25°C and stirring

5.2 Preparation of Working Reagent (WR) For use with cuvettes and Micro titer plates.

NOTE: Solutions are prepared and labeled as per SOP 22702 (Solutions used in BQC)

5.2.1 2 mL of working reagent (WR) is required for each test sample, spiked test sample, and standard to be tested. All samples are tested in duplicate.

5.2.2 Typically, prepare 50 mL of WR for each assay by adding 49 parts of Reagent A to 1 part Reagent B and mix by inversion.

5.2.3 The working reagent is stable for one day at room temperature (RT)

5.3 Direct-Q water, or BDP approved equivalent

5.4 Preparation of standard 100 μg/mL BSA Stock Solution

5.4.1 If a previously-made and unexpired BSA stock solution is not available, make 20 mL of a 0.01% (0.1 mg/mL) BSA stock solution by combining 1mL BSA Standard (at 2.0 mg/mL) from an unopened vial with 19 mL of sterile, nuclease-free water.

5.4.2 Mix the 100 μg/mL BSA stock by repeated inversion for 10 seconds and aliquot 1mL into 20 labeled microcentrifuge tubes. The tube labels must contain the BSA lot number, date of dilution/expiration, and the final BSA concentration (100 μg/mL).

5.4.3 Verify the diluted BSA concentration by performing an A_{280} reading of the diluted stock and a water blank. The measured O.D. 280 should be between 0.0609 and 0.0823 O.D.

5.4.4 Prepared 100 μg/mL BSA standards may be stored at -20°C for up to 1 month before expiration

5.5 Preparation of BSA Working Standard Dilutions

5.5.1 Working Standard Preparation - A set of protein standards is made from the standard stock solution in 5.4.

Table #1

Volume of Stock Standard, 0.1 mg/mL	Volume of Diluent (test sample buffer)	Approximate final Conc. Of Standard
NONE	5.0 mL	0 μg/mL (Blank)
0.050 mL	4.95 mL	1 μg/mL
0.250 mL	4.75 mL	5 μg/mL
0.500 mL	4.5 mL	10 μg/mL
0.750 mL	4.25 mL	15 μg/mL
1 mL	4.0 mL	20 μg/mL
1.25 mL	3.75 mL	25 μg/mL
2.5 mL	2.5 mL	50 μg/mL
5.000 mL	NONE	100 μg/mL

NOTE: A minimum of 5 data points is required for the standard curve. The working BSA standards may be used up to 1 day from the time of dilution if stored at 2-8°C, or for 3 hours if stored at RT.

If there is more than 10% difference in absorbance between standard and/or sample duplicates, the appropriate sample(s) will be rerun, and/or BSA standard points may be dropped at the QC reviewer's discretion. No more than 3 standard replicates may be dropped and no more than one standard point.

5.6 Preparation of the Test and Spiked Test Samples

5.6.1 Test samples require no additional preparation and consist of duplicate reactions each containing 100 µL of the undiluted test sample.

5.6.2 Spiked test samples are prepared in duplicate by mixing 100 µL of the test sample and 500 µL 50 µg/mL BSA standard, and 400 µL of blank diluent. The spiked test sample reactions are performed in order to demonstrate that the test sample buffer and nucleic acids do not interfere significantly with the BCA Assay results. Once the O.D. 562 values for the 25 µg/mL BSA standard and the spiked test samples are determined, calculate the percent recovery of the BSA spike according to Section 8.5.

6.0 Equipment

6.1 Beckman DU 800 Spectrophotometer or equivalent. Refer to ***SOP 22941 Operation of the Beckman Coulter DU 800 Spectrophotometer*** for operating instructions.

6.2 Quartz, plastic, or acrylic cuvettes (full height) are permissible.

6.3 Calibrated Pipettes of the appropriate volume ranges.

6.4 Disposable borosilicate glass culture test tubes, 16 mm x 125 mm or 15 mL conical tubes.

6.5 37°C ± 2°C water bath with tube rack.

7.0 Procedure

7.1 Allow UV/VIS lamp to warm up for at least 15 minutes before using (See ***SOP 22941 Operation of the Beckman Coulter DU 800 Spectrophotometer***) for operating instructions.)

7.2 Select the Single Wavelength setup and set at 562 nm.

7.3 Prepare Standards, Test Samples and Spiked samples according to the chart below (See 5.5 for protein standard solutions.).

Reagents	Standards	Test Samples	Spiked Sample
Standard	1000 µL	0	0
Test sample	0	100 µL	100 µL
Spike with BSA standard (50 µg/mL)	0	0	500 µL
Diluent	0	900 µL	400 µL

7.4 Add 1 mL of working reagent (WR) to all tubes. To mix, vortex each test tube for 15 - 10 seconds and cover with parafilm or cap tube.

7.5 Incubate all samples (including the blank) for 60 minutes (± 2 minutes) in a 37°C ± 2°C water bath, after incubation allow samples to cool to RT for 15 minutes (± 1 minute) before reading the sample absorbance. Alternatively, if a water bath is not available, the samples may instead be incubated for 2 hours (± 4 minutes) at room temperature (25 ± 2°C).

7.6 Blank the spectrophotometer using the test sample buffer.

7.7 Record the absorbance at 562 nm of the standard duplicates starting with the blank and lowest concentrations. Read the test and spiked test samples last. Read all samples as quickly as possible to prevent additional color development in the later samples. The use of individual acrylic/plastic cuvettes for each sample will allow for the quickest collection time.

7.8 Print the spectrophotometric results and immediately continue with the data analysis in order to determine if analysis conditions are met.

8.0 Calculations and Analysis

8.1 See UV/VIS ***SOP 22941 Operation of the Beckman Coulter DU 800 Spectrophotometer,*** for instructions for recording, calculating and printing standard curves and protein concentrations. Calculations may be performed using the read only Microsoft Excel spreadsheet located at: [redacted]. Save the post-analysis results to: Scidata drive (currently < [redacted] >), with a file nomenclature of: <BDPQC request #_SOP22107_Date (MM/DD/YY)_Assay number (if repeated)>.

8.2 Average all replicate O.D. 562 nm measurements for test replicates determine the percent standard deviation between each sample measurement. If the sample duplicates are >10% different from the average duplicate value, they cannot be used. See 3.5.1 for re-assay conditions and the requirements for dropping standard curve points.

8.3 Graph and record the standard curve formula and R-squared value of the averaged BSA standard O.D.'s from 1 μg/mL to 100 μg/mL. The R^2 value for the standard curve (not including dropped points) should be greater than 0.95 for a valid test result.

8.4 Determine and record the test sample and spiked test sample protein concentrations using the standard curve formula as below.

$Y = a_1X + a_0$ Y = absorbance

$X = Y - a_0/a_1$ X = concentration

a_1 = slope

a_0 = absorbance

8.5 Calculate the spike recovery of the BSA spiked test samples by dividing the average calculated spike concentration by the average calculated 25 μg/mL BSA standard concentration. Record the spike recovery percentage on the attached results, Form 22107-01. Spike recoveries percentages <50% or greater than 200% of the 25 μg/mL BSA standard invalidate the assay; see the QC Supervisor for further instructions.

8.6 Note all dropped and retested points on the BCA Results, Form 22107-01

9.0 Documentation

9.1 Print the raw O.D. 562 nm data, graphs, standard curve formula, and R-squared value. Attach all printouts to Attachment I, BCA Results, Form 22107-01.

9.2 Save all electronic analysis records in the BCA Protein folder in the Scidata drive (currently < [redacted] >), with a file nomenclature of: <BDPQC request #_SOP22107_Date (MM/DD/YY)Assay number (if repeated)>.

9.3 Record in the UV/VIS logbook: date, user, lamp time on, lamp time off, file name for samples and standard curve, and any additional comments.

10.0 References and related documents

BCA Protein Assay Reagent Kit Instructions, Part Number 23225, 2003. Further information from Pierce may also be found at: www.piercenet.com.

Attachment 1 SAMPLE BCA Protein Assay Analysis Spreadsheet

11.0 Change Summary

Attachment 1

~ SAMPLE BCA Protein Assay Analysis Spreadsheet ~

BCA Protein Assay Analysis Spreadsheet

Print and attach to SOP022107 Attachment I and QC request form

Please save QC request data to: <H:\BDP Public\QC Files\Molecular Biology\Reports\Assay Reports\BCA Protein\"filename">

where the file name is: "BDPQC request number_SOP022107_Date (MM-DD-YY)_Assay repeat number (if needed)".xls

SOP 022107 QC Operator:_____ Date:_____

Revision 0

Date 1/21/2004 Reviewed By:_____ Date:_____

Standards:		ug/mL	O.D. 562nm	Averaged value	
	Standards	0			*#VALUE!*
		1			
	(BSA)	5		STD Curve Values and Variables (Y-A)/B=X	
		10		B	*#VALUE!* *#VALUE!* A
		15		SE (n)	*#VALUE!* *#VALUE!* SE (n-1)
		20		R^2	*#VALUE!* *#VALUE!* Std. Dev.
		25		F score	*#VALUE!* *#VALUE!* Df
		50		SS (reg)	*#VALUE!* *#VALUE!* SS (resid)
		100			

Test Sample(s) and Spikes:	ID #	O.D. value	Average	ug/rxn	Sample Concentration (ug/ml)
Sample 1 Name:	1				
	1		*0.0000*	*#VALUE!*	*#VALUE!* ug/mL
Lot:	1				*#VALUE!*
	1				
	1		*#DIV/0!*	*#DIV/0!*	
Sample Volume Tested (uL):	1				
	1				
Spike Mass is: ug/rxn	1spike				*Spike Recovery ug/rxn and %*
	1spike		*0.0000*		*#VALUE!* *#VALUE!* *#VALUE!*
	1spike				