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1.0 Purpose

This procedure determines the absorbance (Optical Density) of a protein solution with a UV/VIS Spectrophotometer (A_{280}).

2.0 Scope

This Standard Operating Procedure applies to Process Analytics Personnel who perform fixed wavelength analysis using the UV/VIS spectrophotometer in order to ensure proper and consistent utilization.

3.0 Authority and Responsibility

3.1 The Director of 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 (BQA).

3.3 PA personnel are responsible for 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 Equipment and Materials

4.1 Beckman Coulter DU800 Spectrophotometer, or BDP approved equivalent.

4.2 Computer Software (proprietary).

4.3 Bovine Serum Albumin Standard, BDP PN 30060, or Bovine Gamma Globulin Standard, BDP PN 30059, or BDP approved equivalent.

4.4 PBS buffer, BDP PN 30007, or BDP approved equivalent (i.e., formulation buffer of sample).

4.5 Borosilicate glass culture tubes (12 x 75 mm) BDP PN 20143, and (16 x 125 mm) BDP PN 20144 or BDP approved equivalent.

4.6 Calibrated Pipettors 20-200 μL and 100-1000 μL.

4.7 Pipette tips 250 μL, BDP PN 21767, and 1000 μL, BDP PN 20769, or BDP approved equivalent.

4.8 Gloves, BDP PN 20766 or BDP approved equivalent.

4.9 Vortexer.

4.10 Quartz Cuvettes (1 cm pathlength).

4.11 Disposable Pasteur pipettes, BDP PN 20843 and BDP PN 21107, or BDP approved equivalent.

4.12 Methanol, HPLC grade or better, BDP PN 10115, or BDP approved equivalent.

4.13 Direct-Q water, or BDP approved equivalent.

4.14 Kimwipes EX-L, BDP PN 20091, or BDP approved equivalent.

4.15 Aspirator cuvette cleaner with vacuum line.

5.0 Procedure

5.1 Turn on the power to the spectrophotometer by double clicking on the *DU 800* Spectrophotometer icon. Turn on the UV and Visible lamps by clicking the Visible and UV buttons in the lower left-hand corner. Refer to **SOP 22941, *Operation of Beckman Coulter DU800 Spectrophotometer*.**

5.2 Allow the instrument to warm up (approximately one hour).

5.3 At the Main Screen, select Fixed Wavelength from the drop-down box.

5.4 Select Method A_{280} from the drop-down box.

5.5 Using the Cuvette

5.5.1 Wear gloves or avoid touching the clear front and back of the cuvette.

5.5.2 Wash the cuvettes using the Aspirator cuvette cleaner; first with water, then with Methanol. Make sure to wash the cuvette before placing a new sample in the cuvette.

5.5.3 Wipe the clear sides of the cuvette gently using a Kimwipe before placing into the sample holder.

5.5.4 Make sure the cuvette is filled at least a third (400 μL). If the sample size is small, use a limited volume cuvette (300 μL).

5.5.5 If possible, use the same cuvette for the blank and samples.

5.6 Blanking

5.6.1 Fill the cuvette with PBS, or appropriate buffer provided with the submitted sample.

5.6.2 Place the cuvette into sample compartment number 1 (the furthest away from you) with the transparent side of the cuvette facing towards the lightpath (left and right).

5.6.3 Close the lid before performing the analysis.

5.6.4 Left click the Blank icon to blank the spectrophotometer.

5.6.5 Left click on the Sample ID. Label the sample "Buffer Blank."

5.6.6 Left click on Read Samples.

5.6.7 Remove the cuvette from the sample compartment and wash (refer to step 5.5.2).

NOTE: Because the instrument is blanked using the buffer, when reading the buffer, it should read approximately 0.00 au.

5.7 Standardizing the Instrument

5.7.1 If analyzing antibodies, use Bovine Gamma Globulin (BGG) as the standard. For all other samples, use Bovine Serum Albumin (BSA).

5.7.2 Dilute BSA/BGG to 1 mg/mL (1:2) with PBS or appropriate formulation buffer in a culture tube and vortex slightly; make three separate dilutions for three individual readings. For example, add 200 µL of BSA/BGG standard to 200 µL of PBS or sample buffer in a borosilicate glass culture tube.

5.7.3 Left click on Sample ID to label the standard/buffer; include the dilution factor and/or concentration (i.e., BSA 1mg/mL).

5.7.4 Fill the washed cuvette with standard/buffer prepared in step 5.7.2.

5.7.5 Place the cuvette in the sample compartment number 1 (refer to step 5.6.2).

5.7.6 Left click on Read Samples.

5.7.7 BSA at 1 mg/mL should read approximately 0.7 au and BGG at 1 mg/mL should read approximately 1.3 au. If the results obtained do not fall within 10%, the instrument is out of specification. The BSA acceptable range is 0.63 - 0.77 au and the BGG acceptable range is 1.17 – 1.43 au. Refer to **SOP 22004, *Managing Out-of-Specification Test Results or Unexpected Test Results***, for further proceedings. If the results are within 10% of these values, then proceed with the analysis.

NOTE: The A_{280} values can be divided by the extinction coefficient of the molecule (if known) to give an accurate protein concentration. Otherwise, the result will only be reported as an absorbance value.

5.7.8 Remove the cuvette from the sample compartment and wash (refer to step 5.5.2).

5.7.9 Refill the cuvette with the next sample/standard and repeat the reading (second reading) refer to steps 5.7.4 and 5.7.6.

5.7.10 Repeat step 5.7.9 for the third reading.

5.7.11 Blank the instrument before each sample or standard with the appropriate buffer (refer to 5.6.1 – 5.6.4).

NOTE: There is no need to blank between each reading.

5.8 Samples

5.8.1 Samples, which contain colloidal dispersions, dust, or other particulate matter, should be filtered, centrifuged, or allowed to settle before any measurements are attempted.

5.8.2 Dilute samples in the same fashion as the standard (in triplicate); dilute the sample to obtain an absorbance between 0.2 and 1.0. This may entail trying several dilutions to fit within the desired range.

5.8.3 Read the samples (refer to steps 5.7.3-5.7.11).

5.8.4 Make sure there are a total of three individual readings for each sample.

5.8.5 Repeat this entire section for all samples.

5.8.6 When all the samples have been analyzed, turn the UV and Visible lamp off in the same fashion it was turned on (refer to step 5.1).

NOTE: This procedure can be used for analyzing samples at any wavelength. Select the desired wavelength from the drop-down box.

5.9 Drift

5.9.1 Repeat the standardization section to ensure the instrument has not drifted (3.7). If the results obtained are not within 5% of the initial standardization, Refer to **SOP 22004*, Managing Out-of-Specification Test Results or Unexpected Test Results***, for further proceedings. If the results are within 5%, the analysis is complete.

5.10 Results

5.10.1 After completing the assay, save the data by clicking on the File button and then Headers for Current Run. Fill in the Department, Operator, Product Name, Component Name, Lot Number, and Comments (if the information is provided on the QC Test Request Form). Click OK.

5.10.2 Click the File button and then Save as. From the drop-down menu in the Save in window, select QC Shared on 'Perfection' (I:). Double click on the QC Public folder and then the DU800 Data folder. Save the data by using the QC test request number or the date the assay was performed. For example, QC033333 LMB-2 11-02-06. Click the Save button.

5.10.3 Print out the report by clicking on the File button, Print and then OK. After completing the entire test, click Systems from the top menu and then Exit. Turn off the Lamps.

5.10.4 Average all three absorbance readings and multiply by the dilution factor, to obtain result in Absorbance units. As requested, divide this result by the extinction coefficient to obtain protein concentration in mg/mL.

6.0 Documentation

6.1 Record the results from step 5.10.4 on the BQC Test Request Form 22002-01. Attach the A_{280} printout from step 5.10.3 to the BQC Test Request Form.

6.2 Record the sample preparation on Form 22180-01, A_{280} Sample Preparation. Attach the form to BQC Test Request and submit it for PA and BQA review.

6.3 Record all use of the UV/VIS in the Equipment Logbook, refer to **SOP 21531, *Equipment/Facility Logbooks***.

7.0 References and Related Documents

SOP 21531	*Equipment/Facility Logbooks*
SOP 22004	*Managing Out-of-Specification Test Results or Unexpected Test Results*
SOP 22941	*Operation of Beckman Coulter DU800 Spectrophotometer*
Form 22180-01	*A_{280} Sample Preparation*

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