Core Equipment ID: 9995

Description: Beckman DU 640 Spectrophotometer

Room: B446 (Molecular & Biochemical Core)

Champion:

1.0 Purpose
Standardize the process for control, maintenance, ownership and use of the Beckman DU 640 Spectrophotometer located in B-446 Life Sciences.

1.1 Beckman DU 640 Spectrophotometer Capabilities
The DU Series 600 Spectrophotometer is a microprocessor controlled spectrophotometer intended for use in quantitative and qualitative biological research and industrial procedures that require spectrophotometric measurements in the UV-visible region of the electromagnetic spectrum. The instrument has three standard Routine Measurement modes. They include:

a. Fixed Wavelength – Takes absorbance or transmittance readings at up to 12 wavelengths. Readings at each wavelength can be multiplied by a factor.

b. Wavelength Scan – Performs wavelength scans in absorbance or transmittance. Data are automatically stored for manipulations including Trace, zoom, overlay and tabulate. Calculations include peak pick, valley pick, point pick, first to fourth derivative, log of absorbance, scatter correction, spectral addition, subtraction and multiplication, and net absorbance. Repetitive scanning is also performed in this mode.

c. Kinetics/Time – Calculates the rate of an absorbance versus time reaction with a choice of blank subtraction and graphic display of the data for multiple samples. Data are automatically stored for manipulations including Trace, zoom, overlay and tabulate.

Beckman DU 640 Spectrophotometer Application Capabilities

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The Beckman DU 640 Spectrophotometer has a number of other application modes, including, but not limited to:

a. Protein Analysis – Calculates the protein concentration using the Bradford, Lowry, Biuret, direct UV method, colloidal gold, and bicinchoninate (BCA). Prepares a standard curve using up to 30 standards. The User can choose to add, delete or rerun individual standards based upon a statistical analysis of the standard curve.

b. Nucleic Acid – Determines protein impurity in nucleic acid samples based upon the ratio of readings at two wavelengths with a choice of background correction.

c. Fraction Read/Plot – Collects, plots and tabulates data from a set of related fractions. Reading can be corrected for dilution. After data collection, individual fractions can be added, deleted or rerun. Data are plotted versus either fraction number or volume. Other data obtained for the fractions can be input and plotted with the absorbance data.

d. Enzyme Activity – Calculates the enzyme activity of large numbers of samples.

e. Performance Validation – Provides a simple procedure to verify the performance of an instrument. Tests which are performed include: wavelength accuracy and repeatability, resolution, baseline flatness, noise and stability.

2.0 Reason for Issue
Maintain a document that describes the Standard Operating Procedures that allows for the standard safe and maximal use of the Beckman DU 640 Spectrophotometer within the Pharmacology and Toxicology Core Facilities.

3.0 Process Description
Allows Core Facility Users within the Pharmacology and Toxicology Department to properly and effectively use the Beckman DU 640 Spectrophotometer. The process description details and creates a standard for the use of the Beckman DU 640 Spectrophotometer. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

3.1 SOP: 9995.B446.001 for Beckman DU 640 Spectrophotometer, authored by Janice Thompson, created on April 2, 2009, issued on (insert date).
3.2 SOP: 9995.B446.001 applies to any user and/or trainer of the Beckman DU 640 Spectrophotometer.

3.3 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the Beckman DU 640 Spectrophotometer. All users are responsible for the proper use, according to defined protocol, when using the Beckman DU 640 Spectrophotometer.

   a. New Users must ensure their familiarity with the equipment through the thorough reading of this SOP and the associated Operating Instructions provided by Beckman. The Operating Instructions manual is located in the closed cabinets on the right side of the Core Facilities Room, B446 Life Sciences.

   b. All Users must schedule equipment use in the Equipment Schedule Logbook prior to use. The Equipment Schedule Logbook is located on the desk next to the Beckman DU 640 Spectrophotometer.

   c. All Users must record all equipment use in the Equipment Usage Logbook post-use on the same day as the recorded use. The Logbook is located on the desk next to the Beckman DU 640 Spectrophotometer. Within the Logbook on the current log sheet, Users must record the following: Date, PI, Name, Lab Location, Phone Number, Program Used, Number of Samples, Comments.

   d. Immediately after use, the Beckman DU 640 Spectrophotometer must be cleaned with distilled water and a Kimwipe to remove any chemical spills or residues.

3.4 **Equipment Safety Issues**

   a. When using the cell holder within the Beckman DU 640 Spectrophotometer, care MUST be taken to ensure that it is installed within the unit appropriately. The cell holder is held in place by two metal posts that are screwed into the base. Do not force the posts in. If they are difficult to screw in place, remove it and attempt again, as the metal posts can be bent easily.

   b. The cell holder must be removed when work has been completed. If it remains in the Beckman DU 640 Spectrophotometer, it will interfere with the startup mechanisms the next time the spectrophotometer is started.

3.5 **Quality Measures**
a. Each Use: Each time the Beckman DU 640 Spectrophotometer is used, wipe all chemical spills and residues from both the case and the cell holder. Use only warm water and a damp cloth to clean the Beckman DU 640 Spectrophotometer.

b. Every Six Months: Inspect cables and power cords to ensure their integrity.

3.6 Procedure for Analysis of Protein Samples Using the BCA Protein Assay; Beckman DU 640 Spectrophotometer

Setup

a. While samples are incubating, prepare the Beckman DU 640 Spectrophotometer for use. Open the Beckman DU 640 Spectrophotometer and ensure the cell holder is not in place. If it is, remove it. Close the lid.

b. Turn on the Beckman DU 640 Spectrophotometer using the toggle button located on the rear of the machine, in the lower right corner. Turn on the monitor.

c. Turn on the printer located to the right of the Beckman DU 640 Spectrophotometer.

d. The Beckman DU 640 Spectrophotometer will go through its “Executing Power Up Diagnostics” scan, making a few clicking and whining noises as it does so.

e. When the diagnostic scan is complete, the “Power Up Diagnostic” screen will be displayed. Each test name will have “Pass”, “Fail” or “Not Installed” following it. If any tests “Fail”, turn off the Beckman DU 640 Spectrophotometer and restart it. If all tests “Pass” or are “Not Installed”, close this screen by clicking “Quit”.

f. The Main Window is displayed on the screen. All writing in white can be clicked on to change parameters or advance to another screen.

g. Turn on the visible light by clicking the “[VIS OFF]” writing at the bottom of the window. It will change to “[VIS ON]” to identify the lamp has been turned on.

h. In the “Application” box, click on “Protein”.

i. Read the information on the window that is opened. Ensure it is the BCA Protein Assay that will be used. If it is not, click on the
assay that is displayed and choose the BCA Protein Assay. Ensure the correct number and value of the standards.

j. To change the number of standards, click on the white number next to “Number of Standards”. Select the appropriate number by clicking on it. Click “OK” when finished.

k. To change the value of the standard, click on the white number that is to be changed. Select the appropriate value. Click “OK” when finished.

l. Allow the machine to warm up at least 10 minutes before using.

Sample Analysis

a. When the samples are ready, bring them and disposable cuvettes to the Beckman DU 640 Spectrophotometer. Work on the bench behind the Beckman DU 640 Spectrophotometer (covered in white absorbent paper and next to the sink) when pouring samples into the cuvettes to ensure the Spectrophotometer is not contaminated.

b. Open the lid of the Beckman DU 640 Spectrophotometer and insert the cell holder, with “FRONT” toward the front of the opening. Ensure that the metal posts are properly aligned before screwing them into place.

c. Place the black insert into the cell holder, with “BECKMAN” facing the right side of the spectrophotometer.

d. Add distilled water to a disposable cuvette and place it in the black insert, in the slot farthest from the front of the cell holder. Ensure that the clear sides of the cuvette are facing left to right.

e. Close the lid and click on “Blank” at the bottom of the screen. The “Reading Blank” message will appear on the screen. When it is gone, remove the cuvette from the Beckman DU 640 Spectrophotometer.

f. Pour each of your standards into a disposable cuvette. The Beckman DU 640 Spectrophotometer reads samples from the back to the front, so load the cuvettes into the black insert beginning at the back with your lowest standard amount. This is to coordinate with the standards listed on the screen.

g. Close the lid and press “READ 1” on the left side of the window. The Beckman DU 640 Spectrophotometer will emit some whining noises as it reads the samples. Absorbance measurements will appear in the window as the samples are read. If more than six standards are used, remove the cuvettes from the Beckman DU
640 Spectrophotometer and repeat steps “f” and “g” until all are read.

h. When all standards have been read, press “Display Curve” on the top of the screen. Another window will open with the standard curve displayed in it. Print the curve by clicking “Print Curve” at the top of the screen. A message box will be present on the screen until printing is complete. When complete, close the screen by pressing “Quit” in this window.

i. Click on “Samples” in the upper left corner of the window. A new screen will appear for the sample measurement.

j. In the information at the top of the screen, ensure there are two replicates being tested.

k. Pour each sample, up to six at a time, into its own disposable cuvette. Insert them in the black insert, beginning at the back, and with duplicates next to each other. Close the lid.

l. Press “Read Samples”. Each sample’s individual value, plus the mean for the duplicates, will be displayed on the screen. A soft whining can be heard during this time. Do not open the lid until the “Reading Samples” message is gone from the screen.

m. Open the lid and remove the cuvettes.

n. If more samples are to be read, repeat steps “k” through “m” until all have been read.

o. To print the values, click “Print” on the upper row of the screen.

**Clean Up**

a. When all samples have been read and everything has been printed, clean up the work area.

b. Remove all cuvettes from within the Beckman DU 640 Spectrophotometer.

c. Unscrew the metal posts of the cell holder and remove both the cell holder and the black insert from the Beckman DU 640 Spectrophotometer. Close the lid. Store the cell holder and the black insert on the top of the Beckman DU 640 Spectrophotometer, in its white protective holder.

d. Close the program by clicking “Quit” in the upper right corner of the screen. Do not save your measurements. Click “OK” to exit.
e. Turn off the Beckman DU 640 Spectrophotometer by pressing the toggle switch on the rear of the machine. Turn off the monitor. Turn off the printer.

f. Clean up any spilled liquid using a Kimwipe dampened with water.

g. Record use of the Beckman DU 640 Spectrophotometer in the Equipment Use Logbook.

h. Take all cuvettes, test tubes and used Kimwipes with you to dispose of appropriately.

3.7 Core Materials
Currently, there are no Core Materials provided for the Beckman DU 640 Spectrophotometer.

3.8 User Materials
Users are individually responsible for the compatibility, maintenance and purchase of materials and chemicals used with the Beckman DU 640 Spectrophotometer.

3.9 Use Records
a. Records of Use – All Beckman DU 640 Spectrophotometer use must be recorded. Refer to 3.3c.

b. Error Messages / System Issues – All error messages and system issues must be relayed to the Equipment Champion and the Pharmacology & Toxicology Core Facility Manager. This information must be relayed on the same day as equipment use. Error messages/system issues must be recorded. Refer to 3.3c.

3.10 Resource Index
Beckman DU 640 Spectrophotometer Operating Instructions may be found in the closed cabinets located on the upper right wall of the Core Facilities Room, B446 Life Sciences.

4.0 Competences, Authorization and Training
New Users must receive proper authorization from either the Equipment Champion and/or Pharmacology & Toxicology Core Facility Manager before equipment use. A new User may contact the Equipment Champion or Pharmacology & Toxicology Core Facility Manager to schedule training. Training includes SOP and instrument familiarization and any additional required or specialized training. All Users are individually responsible for current SOP familiarization.
5.0 SOP Performance and Equipment Review

The effectiveness of the SOP#: 9995.B446.001 will be monitored by the Pharmacology & Toxicology Core Facility Manager, Equipment Champion and all Users. Any procedural or qualitative deviations will be reflected within an updated SOP. Any approved User should aptly report any procedural or qualitative issues and/or errors to the Pharmacology & Toxicology Core Facility Manager or Equipment Champion. The Equipment Champion’s name and contact information can be found on the Core Facility Identification Sticker. The Core Facility Identification Sticker is located on the Beckman DU 640 Spectrophotometer. The Core Facility Manager’s contact information may be found on the Core Facility Contact Posting within room B446. Updated SOPs will be published and approved Users will be notified. SOP: 9995.B446.001 review will occur every two years.

5.1 Update Date:
Reason for SOP Change:
New Version #:

5.2 Update Date
Reason for SOP Change
New Version #:

6.0 Definitions

Any standard defined term specifically used in the Pharmacology and Toxicology Core Facilities’ SOP. These are terms that carry a unique Core Facility definition that is pertinent to proper SOP understanding and implementation. A list of terms and definitions is maintained to allow Users a quick and efficient reference. Listed below are common Pharmacology and Toxicology Core Facility terms. New common terms are to be approved and added, with definition, to the term reference guide.

a. **SOP** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance and ownership of the Beckman DU 640 Spectrophotometer. The SOP number stand for (xxx . xxx . xxx) equipment number . room number . SOP version number.

b. **Originator / Author** The individual representing the Pharmacology and Toxicology Core Facilities that created SOP: 9995.B446.001.

c. **Stakeholder** Any individual that uses the Beckman DU 640 Spectrophotometer, including the Pharmacology and Toxicology Core Facilities Department.

d. **New User** An individual who has not completed the requirements of section 4.0.
e. **Approved User** An individual who uses or performs the task of which is the subject of the SOP and has fulfilled section 4.0. This title may only be given by the Equipment Champion and / or the Pharmacology and Toxicology Core Facilities Manager.

f. **Champion** An individual whose direct expertise with the Beckman DU 640 Spectrophotometer has been recognized by the Pharmacology and Toxicology Core Facilities Committee. The Pharmacology and Toxicology Core Facility Committee may only award the champion title.

### 7.0 Appendix

The below signatures and dates are required for full SOP approval and implementation.

This SOP was written by:  
Janice Thompson  

This SOP was reviewed by:  
Dr. Stephanie Watts  

This SOP was authorized by:  
Dr. J.R. Haywood  

Issue Date:  