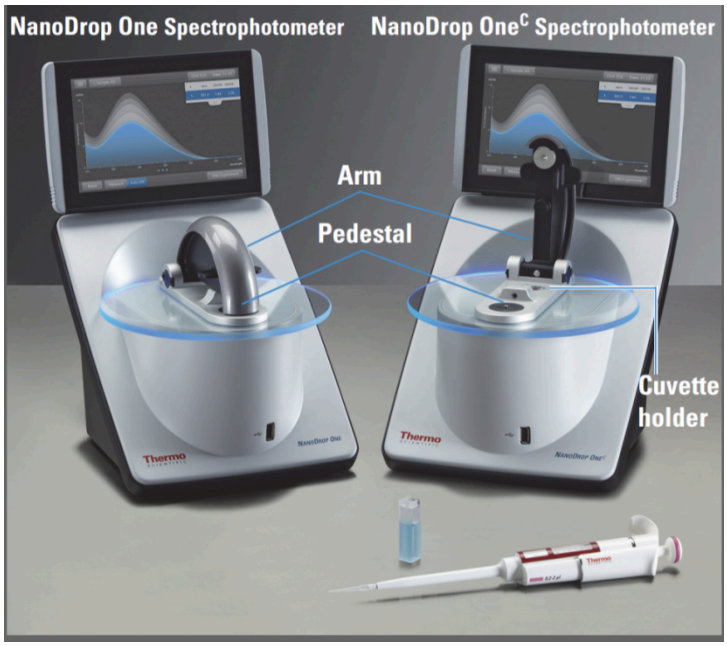
University of Minnesota Nano Center Standard Operating Procedure

Equipment Name: NanoDrop Microvolume UV-Vis Spectrophotometer
Model: NanoDrop One^{C}
Location: PAN 181/185
Version Number: 2
Last Revision Date: 11/12/2019
Last Revised by: M. Johnson

A. Introduction

1. **Tool Description.** The NanoDrop One^{C} is a compact, stand-alone UV-Visible spectrophotometer that can analyze small volumes of nucleic acids, proteins, and non-biological samples. It can analyze samples using a micro-volume pedestal (1-2 µL) or a standard cuvette (1-3 mL). It also has a 37 ˚C heater option and a micro-stirring option to ensure sample homogeneity and support kinetic studies.

2. **Tool Components**. Figure 1 shows a front view of the NanoDrop One^{C}. Key components visible in this view include the arm and pedestal (for microliter volumes of sample) and the cuvette holder (for 1-2 ml samples).



3. **Safety**. Do not attempt to look at the UV light source during operation.

4. **Restrictions/requirements**
   - Use solvents that are compatible with the instrument (see Hazardous Materials)
   - Do not use hydrofluoric acid (HF) on the pedestals.
   - Do not use a squirt or spray bottle on or near the instrument as liquids will flow into

the instrument and may cause permanent damage.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, please notify MNC staff immediately.

5. **Hazardous Materials.** Many standard spectroscopy methods are based on the use of solvents. Most solvents typically used in life science laboratories are compatible with the NanoDrop spectrophotometer. However, high vapor pressure solvents may not be conducive to small volume measurements, due to rapid evaporation. To measure samples in such solvents, use a cuvette.

   The following solvents are compatible for use on the pedestals of the NanoDrop:

● methanol	● ethanol	● n-propanol
● isopropanol	● butanol	● acetone
● ether	● chloroform	● carbon tetrachloride
● DMSO	● DMF	● acetonitrile
● THF	● toluene	● hexane
● benzene	● sodium hydroxide	● sodium hypochlorite (bleach)
● dilute HCl	● dilute HNO_3	● dilute acetic acid

   All corrosive solvents must be wiped from the pedestal immediately upon completion of a measurement. Avoid prolonged exposure of the pedestal and surrounding diaphragm to HCl, alcohol, bleach, acetone or other solvents, which may damage the diaphragm seal adhesive. If the seal comes loose notify MNC staff immediately.

6. **Required Facilities:** 120 V outlet

7. **Theory of Operation.** A UV-Vis spectrophotometer measures a sample's absorption of light in the visible to near ultraviolet range of the electromagnetic spectrum. The absorption spectrum can be used to determine the concentration of absorbers in a solution or dispersion, as well as suggest the particle size or the chemical identity of the absorber. In the NanoDrop, the wavelength range is 200 to 900 nm.

   The NanoDrop UV-Vis spectrophotometer can be used for several different types of analysis.
   - Concentration of DNA in a sample (both single- and double-stranded)
   - Concentration of RNA in a sample
   - Concentration of nucleic acids labeled with a fluorescent dye
   - Total protein concentration
   - Monitoring the growth rate of microbial cell cultures
   - Particle size of quantum dot nanoparticles
   - Standard spectrophotometric measurements of solutions and particle dispersions

   For details on how to carry out each of these measurements, consult in the NanoDrop User's Manual, located on the NanoDrop computer.

B. Using the NanoDrop One[C]

1. **Equipment Setup**
    a. Begin a new entry on the tool log sheet. List your name, the date, the materials you are working with, and any issues you encountered during your run.
    b. The NanoDrop should be found in an ON condition. If it is not, turn it on using the on/off switch located at the back of the machine.
    c. Secure supplies
        i. For small volume samples, have a supply of DI or distilled water for cleaning the pedestal and a micropipettor (able to deliver down to 1 uL of liquid) with appropriate pipet tips
        ii. For larger volume samples, have a supply of plastic, glass, or quartz cuvettes. Cuvette material must be chosen based on your sample solvent.

2. **Select an Analysis.** All commands to the NanoDrop are given using the instrument touch screen. The home screen on the NanoDrop offers the following classes of experiments, with subtypes listed under each class.
    a. Nucleic Acids
        - double stranded DNA (dsDNA)
        - single stranded DNA (ssDNA)
        - RNA
        - Microarray
        - Oligo DNA
        - Oligo RNA
    b. Proteins
        - A280
        - A205
        - Labels
        - BCA
        - Bradford
        - Lowry
        - Pierce 660
    c. OD600: measures the concentration of microbial cultures using scattered light at a fixed wavelength of 600nm.
    d. Custom: for spectrophotometric measurements of solutions and particle dispersions over a user-defined wavelength range.
        - Standard UV-Vis absorption
        - User-defined custom methods

3. **Running a sample: Microliter volume.** In this mode the sample is placed between the arm tip and the pedestal. Enough liquid must be placed on the lower pedestal so that a meniscus is formed when the arm is lowered. If there is insufficient liquid to maintain a meniscus, the NanoDrop will return an error warning.
    a. Raise the arm and clean the pedestal with DI water and a lint-free lab wipe.
    b. Run a blank measurement with 1-2 uL of clean, particle free solvent, using the same solvent that makes up your sample (must be free of any analyte).
    c. Clean both the pedestal and the arm by wiping with a dry lab wipe.
    d. Pipette 1-2 uL of sample onto the pedestal
    e. Lower the arm. The analysis will begin automatically.
    f. After results are obtained, they will be displayed on the touch screen.
    g. Raise the arm and clean both the pedestal and arm by wiping with a dry lab wipe.

4. **Running a sample: Milliliter volume.** This mode is optimal for lower concentration analytes that require a longer pathlength to get a usable absorption signal. The sample is

placed in a cuvette, either plastic, glass, or quartz. Standard 10x10x45mm acrylic cuvettes are available in the lab. Do not use these plastic cuvettes with any solvent that may dissolve or swell the plastic. Consult with MNC staff if you are working with solvents.

5. **Data Analysis.** The NanoDrop will display the absorption spectrum after each measurement. Use of NanoDrop One viewer…

6. **Exporting your data.** ….

C. Tool Shutdown

1. When your work is complete:
2. Clean the pedestals with DI water and a lab wipe
3. Discard any used cuvettes, pipet tips, etc.
4. Leave the NanoDrop on
5. Close the NanoDrop Viewer program and log off the computer
6. Be sure to complete an entry in the log book.

Protein Analyses Methods

1. **Protein BCA Assay**: The Protein BCA assay uses bicinchoninic acid (BCA) as a colorimetric detection reagent for Cu^{+1} which is formed when Cu^{+2} is reduced by certain proteins in an alkaline environment. A purple reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm and uses a standard curve to calculate protein concentration. A single-point baseline correction is applied, using the absorbance value at 750 nm.

    - This application is useful for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 290 nm, which rules out direct protein measurements at 280 nm or 205 nm. ***Detailed instructions on performing a Protein BCA Assay with the NanoDrop One^{C} can be found beginning on page 93 of the Manual.***

2. **Protein Bradford Assay**: The Protein Bradford assay uses the protein-induced absorbance shift of Coomassie Blue dye as a colorimetric detection reagent to determine total protein concentration. The bound protein-dye complex is measured at 595 nm and uses a standard curve to calculate protein concentration. A single-point baseline correction is applied using the absorbance value at 750 nm.

    - This application is useful for measuring dilute protein solutions that require lower detection sensitivity or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm, which rules out direct protein measurement at 280 nm or 205 nm. ***Detailed instructions on performing a Protein Bradford Assay with the NanoDrop One^{C} can be found beginning on page 103 of the Manual.***

NOTE: To maximize reliability with the Protein Bradford assay:

a) **Work quickly and do not allow prepared standards or samples to sit longer than necessary**. Coomassie dye-dye and Coomassie dye-protein aggregates can form particulates with increasing development time, resulting in significant fluctuations in absorbance readings.

b) **Measure standards and samples in triplicate** using a new aliquot for each measurement. For pedestal measurements, the total analyte (protein-dye) signal at 595 nm is limited to ~0-0.150A due to the pedestal's 1.0 mm pathlength, the Coomassie concentration, and the acidic pH.

3. **Protein Lowry Assay**: The Protein Lowry assay uses Folin-Ciocalteu as a colorimetric detection reagent to determine total protein concentration and involves the reaction of protein with cupric sulfate in alkaline solution, resulting in the formation of tetradentate copper-protein complexes. The Folin-Ciocalteu reagent is effectively reduced in proportion to the chelated copper-complexes. The water-soluble blue reaction product is measured at 650 nm and uses a standard curve to calculate protein concentration. A single-point baseline correction is applied using the absorbance value at 405 nm.

   - This application is an alternative to other colorimetric applications for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm. This application measures absorbance at 650 nm and uses a standard curve to calculate protein concentration. ***Detailed instructions on performing a Protein Lowry Assay with the NanoDrop One^C can be found beginning on page 112 of the Manual.***

4. **Protein Pierce 660 Assay**: The Protein Pierce 660 assay uses a proprietary protein biding material as a colorimetric detection reagent to determine total protein concentration and is based on the binding of a proprietary dye-metal complex to protein in acidic conditions that cause a shift in the dye's absorption maximum, which is measured at 660 nm. The dye-metal complex is reddish-brown and changes to green upon protein binding. The color change is produced by deprotonation of the dye at low pH facilitated by interactions with positively charged amino acid groups in proteins. The color produced in the assay is stable and increases in proportion to the broad range of increasing protein concentrations. The dye interacts mainly with basic residues in proteins such as histidine, arginine, and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine. The reaction product is measured at 660 nm and baseline-corrected using the absorbance value at 750 nm. ***Detailed instructions on performing a Protein Pierce 660 Assay with the NanoDrop One^C can be found beginning on page 120 of the Manual.***

   - An optional Ionic Detergent Compatibility reagent (IDCR) may be added to the assay reagent to increase compatibility with high amounts of ionic detergents, including Lammeli SDS sample buffer with bromophenol blue. The IDCR dissolves completely by thorough mixing and has no effect on the assay.

Microbial Cell Culture Analysis Method

Monitoring OD600: The growth rate of bacterial or other microbial cell cultures can be achieved by measuring the optical density (absorbance) of the culture growth media at 600 nm. The Beer-Lambert equation and user-entered conversion factor are used to correlate absorption with concentration. Reported concentration values can be used to identify the phase of cultured cell populations, e.g, log or exponential and stationary. The OD600 application reports cell concentration in cells/mL. A single-point absorbance correction can be used. This application does not require a standard curve.

The OD600 application measures light transmission and uses that value to calculate absorbance. In spectroscopy, transmitted light is defined as any light that is not absorbed by, reflected from and scattered off of a sample. In the case of living cells, most of the incident light is transmitted through the sample rather than scattered, reflected or absorbed. The amount of scattered light is low and can vary from instrument to instrument. As a result, calculated absorbance readings are typically very low. The calculated absorbance values are used to determine the density of cells in solution in cells/mL. The physical concepts and formulas that relate optical properties of living cells to concentration include:

- Cells, which have a different index of refraction from the surrounding medium, randomly reflect and scatter light out of the incident light path. The amount of scattering is proportional to the density of cells in the sample.
- The Beer's Law equation is used to relate absorbance to concentration.
- For cuvette reading with the NanoDrop One^{C} instrument, accurate absorbance readings are typically in the range between 0.04A and 1.5A. Serial dilutions of the sample are usually needed to bring the absorbance readings within this range.
- All measurements should be made on the same type of spectrophotometer and method (i.e. pedestal vs. cuvette) as the amount of scattered light captured varies based on the optical configuration. When using a different spectrophotometer or method, calculate and apply a conversion factor to the reported results. For example, to compare OD readings using the pedestal vs. a cuvette, a conversion factor can be calculated as follows:

  Conversion factor = Cuvette OD/Pedestal OD

Detailed instructions on performing an OD600 Application with the NanoDrop One^{C} can be found beginning on page 120 of the Manual.