Title: The Operation of the NanoDrop ND1000 Spectrophotometer

SOP Number: 22946

Revision Number: 02

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[redacted]

Originator/Date:

Approval /Date:

Approval /Date:

Table of Contents

1.0 Purpose

This document describes the operation, and maintenance of the Nanodrop ND-1000 full-spectrum (220-750nm) UVN is spectrophotometer.

2.0 Scope

The NanoDrop ND-1000 which measure samples volumes of 1 - 2 µL will be utilized for accurate and reproducible measurement of the following:

- Nucleic acid concentration and purity up to 3500 ng/µL (dsDNA)
- Purified protein analysis (A2so) up to 100 mg/ml (BSA).

Due to its limited sample volume capacity, the NanoDrop ND-1000 will be routinely utilized for in-process assessments.

Exception: Only when alternative measurement methods are not feasible or practical due to the limited availability of test samples as determined by the Project Scientist and Process Analytics/Quality Control (PA/QC) Manager may the NanoDrop ND1000 be used for determining COA-reported release values.

This instrument will be operated by trained PA/QC personnel.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

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

3.3 PA/QC personnel are responsible for the performance of this procedure.

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

3.5 BQA is responsible for quality oversight of this operation.

4.0 Equipment and Materials

4.1 NanoDrop ND-1000 UV/Vis Spectrophotometer; Software ND1000, Version 3.2.1.

4.2 Milli-Q Water or BDP approved equivalent.

4.3 Bleach, BDP PN 10579 or BDP approved equivalent.

4.4 Micropipettes: 2.5 µL, 10 µL.

4.5 Pipette tips with aerosol barriers: 0.1-10 uL, BDP PN 20335 or BDP approved equivalent.

4.6 Kimwipes, BDP PN 20091 or BDP approved equivalent.

5.0 Reagents and Standards

5.1 Lambda DNA Standard (Component C) 100 ug/mL in TE Buffer, BDP PN 30170 or approved equivalent. The storage temperature is 2°C-8°C, but may be frozen (-15°C to -25°C) for long-term storage.

5.2 Bovine Serum Albumin Standard (BSA), 2 mg/mL, BDP PN 30060 or an approved equivalent. This is stored at room temperature.

5.3 Bovine Gamma Globulin (BgG) Standard, 2 mg/mL, BDP PN 30059 or an approved equivalent. This is stored at room temperature.

5.4 CF-1 calibration fluid, BDP PN 30714. This is stored at room temperature.

6.0 Procedure

6.1 Before using the NanoDrop ND-1000, ensure that both the upper and lower pedestals are clean by wiping each with a soft laboratory wipe, such as a Kimwipe.

6.2 Use the same pipette to dispense individual samples on the pedestal for a given series or session of measurements.

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

6.3 With the sampling arm of the instrument in the down position, select the NanoDrop software "ND-1000 V.3.2.1" on the main menu to access the software.

6.4 Select the desired analysis application module from the array of Application Modules on the screen as in Figure 1.

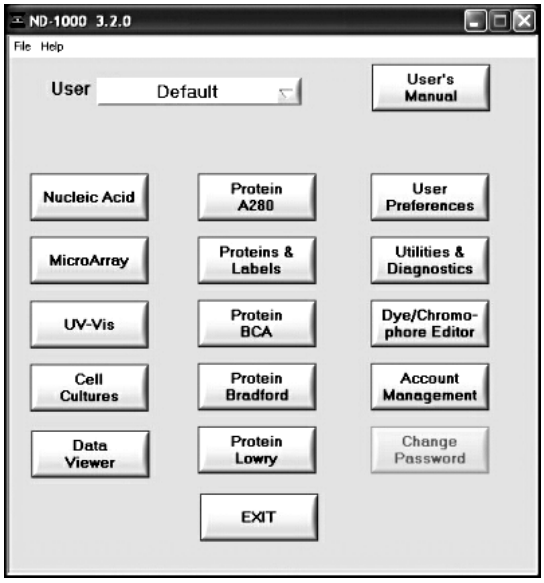

Figure1 Application Modules

6.5 To initialize the instrument, lift up the sampling arm, load 2 µL of Milli-Q water or equivalent, onto the measurement pedestal, lower the sample arm, then select **"OK"** (See Figure 2). Wait until the message **"Initializing the Spectrometer – please wait"** disappears before commencing use of the instrument for measurement.

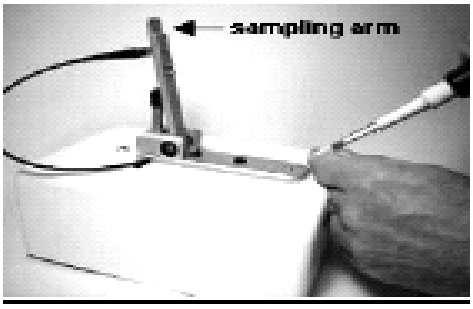

Figure 2 Loading of the Sample

6.6 Begin the measurement session with a Blanking Cycle. The system will not perform measurements without prior blanking. Perform the following:

6.6.1 In the "Sample ID" field indicate "blank" as well as a brief description of blanking buffer/solution.

6.6.2 Load a blank sample (buffer/solvent/carrier liquid) onto the lower measurement pedestal and place the sampling arm in the down position.

6.6.3 Click "Blank" on the screen or depress the **F3** key. Measurement will be completed in approximately 10 seconds.

6.6.4 Upon completion, use a soft wipe to remove the blanking buffer from both pedestals.

Caution: Do not use the "Re-blank" button. Should blanking be again required, perform Step 6.6.2 through 6.6.4 using the appropriate blanking buffer.

6.6.5 Analyze 1 – 2 µL of Milli-Q, or equivalent water as though it were a sample by clicking the "Measure" button, or depressing the F1 key. In the "Sample ID" field, indicate "Water."

6.6.6 Repeat Steps 6.6.1 through 6.6.5 for 3-4 times until a spectrum of relatively flat baseline is obtained. To observe the progression of the baseline, set the Overlay Control to "Accumulate plots until clear."

NOTE: Before loading each sample onto the measurement pedestal, ensure:

(1) Both upper and lower pedestals are clean and free of carryover material.

(2) The sample is homogeneous.

6.7 Common Procedures for Nucleic Acids & Protein Modules

6.7.1 Upon completion of the blanking session the NanoDrop is ready for making measurements. Prior to measuring each sample perform the following:

1. Clean the upper and lower pedestals with a kimwipe.
2. Enter the sample description in the **Sample ID** field.
3. Pipette the sample onto the lower measuring pedestal.
4. Lower the sampling arm on the measuring pedestal so that a column of fluid could be formed.

6.7.2 Click **Measure**, or depress **F1** to perform the measurement. After 10-15 seconds, the window pertaining to the selected module will indicate the appropriate measured values such as $A_{260/280}$, the concentration of ng/µL or µg/mL values based on absorbance at 280 nm, or mg/mL of protein.

6.7.3 Measure each sample at least three times for CGMP analyses, or at least twice for non-CGMP analyses.

6.7.4 After measuring all samples, select **Show Report (F7)** to view the report. In the ND-1000 Data Viewer window perform the following:

6.7.4.1 Enter the Report Name in the designated box. This will consist of the PA/QC # followed by initials of the operator.

6.7.4.2 Confirm that the Test Type is indicated.

6.7.4.3 To save the file select Report →Save Report → Under Report type, choose Full Report. Save in "H:\5PA\PAOnly\QCPublic\NanoDrop 1000 Data" under a filename consisting of the QC number and operator's initials as the suffix.

6.7.4.4 Return to the Analysis window by selecting Exit.

6.7.5 In the window of the analysis application module, select **Print Report (F5)** to print the report.

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

6.8 Nucleic Acids Module

6.8.1 Sample Volume may range from 1 µL to 2 µL. The limit of detection is 2 ng/µL. Approximate upper limits for undiluted samples are 3700 ng/µL (dsDNA,) 3000 ng/µL (RNA), and 2400 ng/µL (ssDNA).

6.8.2 Use the cursor to select the type of sample to be measured. Select "**DNA-50**" for dsDNA, "**RNA-40**" for RNA, "**ssDNA-33**" for ssDNA or "Other" for other nucleic acid. The default is DNA-50. (When "other" is selected, an analysis constant between15-150 may be added.)

6.8.3 Select the wavelength (**λ**) of interest if needed, and corresponding absorbance by moving the cursor or using the up/down arrow keys to the left of the wavelength box.

6.8.4 Prior to analyzing the nucleic acid test samples, perform a pre-test Assessment of the instrument to confirm its accuracy by making three (3) measurements of 1.5 uL of 100 µg/mL Lambda DNA Standard at 260 nm absorbance

6.8.4.1 The acceptance criterion is an average concentration of 100 ± 10 ng/µL based on the three (3) readings at 260 nm absorbance.

6.8.4.2 If the standard fails to meet the set criterion, inform the supervisor.

6.8.5 Perform Steps 6.7.1 through 6.7.3 to execute the measurements.

6.8.6 After analysis of all test samples, perform a post-test assessment of the instrument by repeating Step 6.8.4.

6.8.7 Perform Steps 6.7.4 through 6.7.5 to save and generate the report.

6.9 Protein A_{280} Measurement

6.9.1 A sample volume of 2 µL is required. The limit of detection is 0.1 µg/mL. The upper limit is 100 mg/mL. At higher concentrations this system switches to a 0.2 nm pathlength. However, it displays and records concentrations at the equivalent of 10 nm pathlength.

6.9.2 On the Protein A_{280} screen, select the appropriate sample type (See Figure 3) from the available menu by scrolling the up/down arrow beside the Sample Type box or clicking the box itself.

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

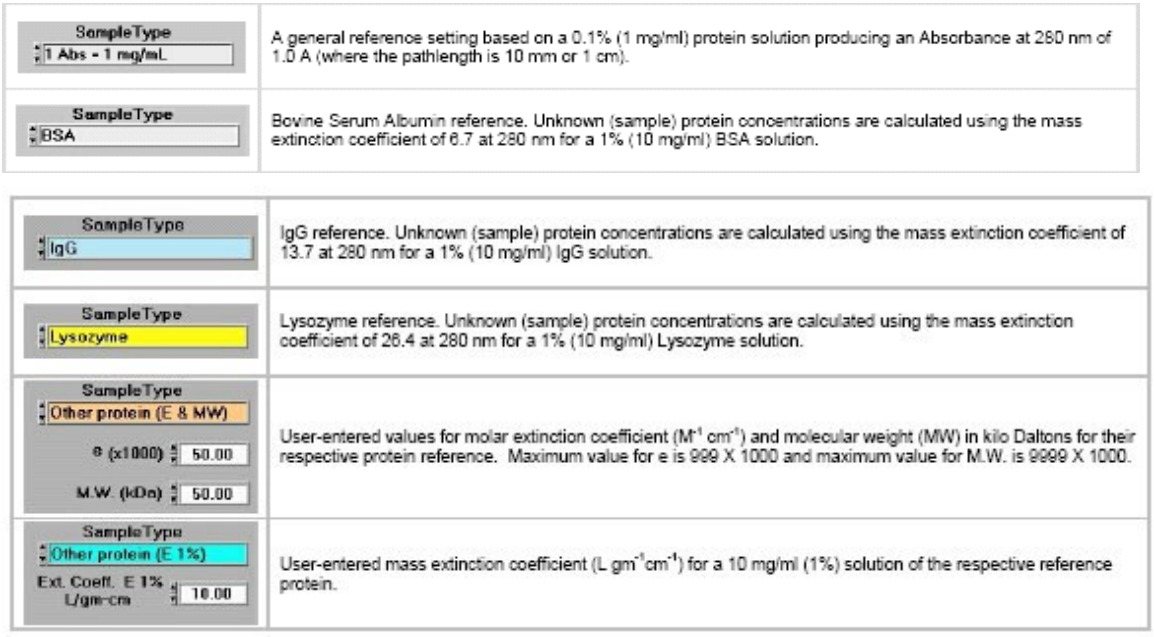

Figure 3. Protein A280 Sample Types

6.9.2.1 The appropriate extinction coefficients are built into the programs of BSA, IgG and Lysozyme Sample Types. When necessary, enter the molar extinction coefficient and molecular weight. All user-entries made will be printed on the report.

6.9.3 Prior to making measurement of the Test Samples, perform a Pre-test assessment of the instrument to confirm its accuracy by making three (3) measurements of 2 uL of 2 mg/mL BSA or BgG Standard at 280 nm absorbance.

6.9.3.1 Use the BgG Standard for antibodies, and BSA Standard for other protein. Criterion for acceptance of this assessment is an average concentration of 2 ± 0.2 mg/mL based on the three readings at A_{280}.

6.9.3.2 If the standard fails to meet the set criterion, inform the supervisor.

6.9.4 Between the measurement of samples due to the presence of surfactants or detergent, the measurement pedestal may become “un-conditioned,” and prevent the formation of the liquid column. Recondition both pedestals by wiping (buffing) their surfaces 15-25 times with a clean dry wipe.

6.9.5 Perform steps 6.7 through 6.7.3 to execute the measurements.

6.9.6 After analysis of all test samples, perform a post-test assessment of the instrument by repeating Step 6.9.3.

6.9.7 Perform Steps 6.7.4 through 6.7.5 to save and generate the report.

6.10 The raw data will be captured on the Report in the format of Attachment 3, produced by selection **Print Report (F5)**.

6.10.1 If the sample had been diluted prior to analysis, complete the appropriate fields of Form 22946-02, Final Concentration Worksheet, an Excel template located in

[redacted] . This is a Rea -on y Ie.

6.10.2 2 After the data have been entered in the template, save the excel file under the newfile name consisting of Form 22946-02, QC number and the operator's initials as the suffix. Example: Form 22946-02 QC01234 _DD.

6.11 The operator will sign the printed report beside the indicated (printed) filename.

7.0 Maintenance

7.1 In addition to wiping the upper and lower pedestals between each sample measurement, upon completion of each series of sample measurements the pedestals of the NanoDrop ND-1000 Spectrophotometer must be cleaned with de-ionized water better.

7.2 Perform a calibration check of the pathlength at least once every six months using a single vial of CF-1 Calibration Fluid as follows:

7.2.1 Ensure the measurement pedestals are clean and that a 1 µL water sample "bead" up on the lower pedestal.

7.2.2 Open the ND-1000 Calibration Check Software by selecting **NanoDrop - Utilities - ND-1000 Calibration Check V3.1.2.**

7.2.3 Follow the prompts in the Customer Guide text box of the software.

7.2.3.1 Enter the Target Absorbance found on the CF-1 vial as directed in the image below.

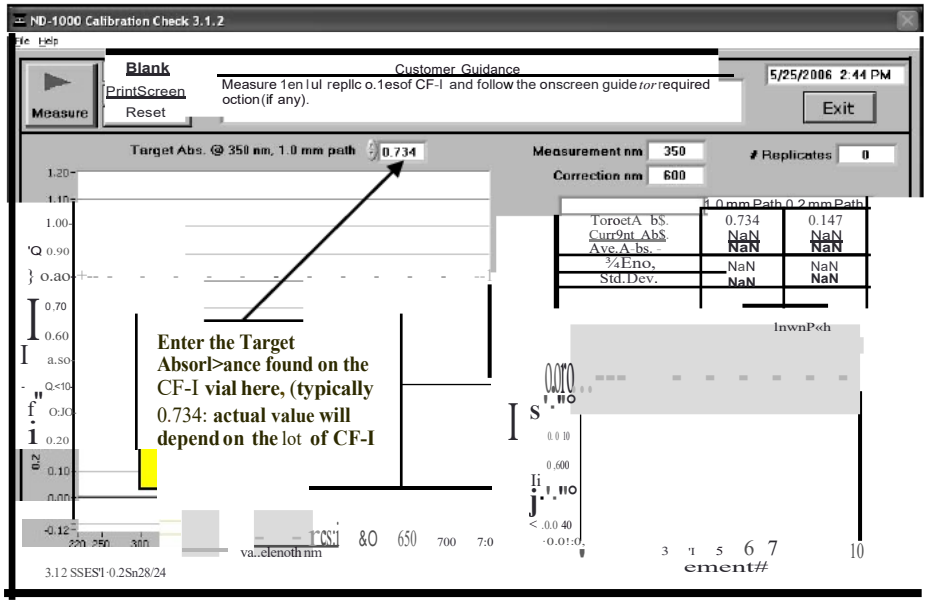

Figure 5: NanoDrop Calibration On-Screen Display

7.2.3.2 Add 1 µL Direct Q or better water, and select "Blank."

7.2.3.3 Vigorously shake the ampule of the CF-1 Calibration Fluid to ensure thorough mixture of the solution before opening. Confirm that the Calibration Fluid is collected in the bottom section of the ampule.

7.2.3.4 Open the ampule, and dispense individual 1µL samples on the lower pedestal.

NOTE: The CF-1 Calibration Fluid must be used within one hour of opening the vial. Exposure to the environment or transferring of the fluid to another container may cause a significant concentration change.

7.2.3.5 Follow the on-screen prompts in the Customer Guidance text box to measure ten (10) replicates.

7.2.3.6 After the tenth measurement, results of the calibration check will be displayed on-screen in the Customer Guidance text box. If the instrument does not pass the calibration check using the 1 µL samples, immediately re-run the procedure again but using 2 µL as in Step 7.2.3.5.

7.2.3.7 Print a copy of the results of the calibration check by clicking the **Print Screen** button. Place a copy in the pertinent logbook.

7.2.3.8 Should recalibration be unsuccessful, inform the supervisor.

8.0 Documentation

8.1 Document the use maintenance, and/or calibration of this instrument on the pertinent logbook per ***SOP 21531 - Equipment Logs***.

8.2 All documentation must be performed in accordance with ***SOP 21409 - Good Documentation Practices***.

8.3 Record the equipment, reagents, and supplies used on Form 22946-01 and attach it to the pertinent QC Request form, Form 22002-01.

8.4 Attach all system-generated print-outs from analyses performed to the documentation or pertinent QC test request form, Form 22002-01.

8.5 Complete the pertinent sections of Form 22946-02, and attach it to documentation or the QC test request, Form 22002-01, if applicable.

8.6 If applicable, record the results of the assay on Form 22002-01.

9.0 References and Related Documents

9.1 **SOP 21409** *Good Documentation Practices*

9.2 **SOP 21531** *Equipment Logs*

9.3 NanoDrop ND-1000 Spectrophotometer V3.2 User's Manual, Revision 1/2006

9.4 <http://www.nanodrop.com/pdf/ND-1000-Calibration-Check.pdf>

10.0 Attachments

10.1 **Attachment 1** Form 22946-01, Equipment, Reagents and Supplies

10.2 **Attachment 2** Form 22946-02, Assay Validation & Final Concentration Worksheet

10.3 **Attachment 3** Prototype of a NanoDrop ND-1000 Report

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

Attachment 1

Form 22946-01, Equipment, Reagents and Supplies

FNLCR, BDP
Form No.: 22946-01
SOP No.: 22946
Revision 02: AUG 04 2017

Equipment, Reagent and Supplies

QC No._____

Description	Equipment MEF or BDP PN or SERIAL No.	Lot Number	Expiry Date or Cal. Due Date
NanoDrop ND-1000			
1.5 mL Microcentrifuge tubes			
___µL Pipette tips			
___µL Pipette tips			
___µL Pipette tips			
___mL pipette (serological)			
Micropipette ___µL			
Micropipette ___µL			
Forceps (sterile)			
Calibration Fluid (CF-1)			
Diluent/Blanking Solution:			
Water:			
BSA / BgG / Lambda DNA Standard (Circle one)			

Recorded By:_____ Date:_____

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

Attachment 2
Form 22946-02, Assay Validation & Final Concentration Worksheet

FNLCR, BDP
Form No.: 22946-02
SOP No.: 22946
Revision 02: AUG 04 2017

Assay Validation & Final Concentration Worksheet

QC #_____

Project #_____

	Sample ID (Per Report)	**Triplicate Measurement** (/)			**Ave Conc.** (/)	**Dilution Factor**	**Final Conc.** (/)	**Total Volume** ( )	**Recovery** ( )
1					#DIV/0!		#DIV/0!		
2					#DIV/0!		#DIV/0!		
3					#DIV/0!		#DIV/0!		
4					#DIV/0!		#DIV/0!		
5					#DIV/0!		#DIV/0!		
6					#DIV/0!		#DIV/0!		
7					#DIV/0!		#DIV/0!		
8					#DIV/0!		#DIV/0!		
9					#DIV/0!		#DIV/0!		
10					#DIV/0!		#DIV/0!		
11					#DIV/0!		#DIV/0!		
12					#DIV/0!		#DIV/0!		

**Insert appropriate units of measurement

Acceptance Criteria Met? YES NO (Circle one)

Comments: _____

Performed By: _____ Date: _____

Reviewed By: _____ Date: _____

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

Attachment 3
Prototype of a NanoDrop ND-1000 Report

Report: QC0333962JT | Test Type: Nucleic Acid | Date/time: 2/7/2007 7:18 PM | Page #: 1

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
Blanking Session H20	Default	2/7/2007	6:11 PM	-279.27	-6.465	NaN	NaN	NaN	50.00	230	NaN	52.541
Blanking Session H20	Default	2/7/2007	6:14 PM	-1.42	-0.028	-0.003	11.37	0.04	50.00	230	-0.646	-0.008
Blanking Session H20-2	Default	2/7/2007	6:16 PM	-1.75	-0.035	-0.020	1.74	0.05	50.00	230	-0.688	0.013
Blanking Session H20-3	Default	2/7/2007	6:19 PM	-1.35	-0.027	-0.012	2.24	0.04	50.00	230	-0.680	0.032
Blanking Session H20-4	Default	2/7/2007	6:40 PM	-6.58	-0.132	-0.093	1.41	0.18	50.00	230	-0.716	-0.660
Blanking Session H20-4	Default	2/7/2007	6:42 PM	-6.73	-0.135	-0.079	1.71	0.19	50.00	230	-0.711	-0.673
Blanking Session H20-5	Default	2/7/2007	6:46 PM	-3.88	-0.078	-0.050	1.54	0.11	50.00	230	-0.708	-0.020
QC0333962-01	Default	2/7/2007	6:48 PM	13.82	0.276	0.149	1.85	0.91	50.00	230	0.303	0.012
QC0333962-01	Default	2/7/2007	6:51 PM	13.53	0.271	0.146	1.85	1.40	50.00	230	0.193	0.002
QC0333962-01	Default	2/7/2007	6:52 PM	13.84	0.277	0.176	1.57	1.39	50.00	230	0.199	0.005
QC0333962-02	Default	2/7/2007	6:54 PM	8.84	0.197	0.123	1.61	1.48	50.00	230	0.133	-0.392
QC0333962-02	Default	2/7/2007	6:55 PM	18.78	0.376	0.233	1.61	0.80	50.00	230	0.417	0.126
QC0333962-02	Default	2/7/2007	6:57 PM	13.48	0.270	0.158	1.71	1.34	50.00	230	0.200	0.100
QC0333962-02	Default	2/7/2007	6:58 PM	12.89	0.258	0.122	2.12	1.40	50.00	230	0.184	0.004
QC0333962-03	Default	2/7/2007	7:02 PM	12.68	0.254	0.158	1.61	1.40	50.00	230	0.182	0.019
QC0333962-03	Default	2/7/2007	7:03 PM	12.25	0.245	0.105	2.33	1.40	50.00	230	0.175	0.010
QC0333962-03	Default	2/7/2007	7:04 PM	12.14	0.243	0.135	1.80	1.40	50.00	230	0.173	0.007
QC0333962-04	Default	2/7/2007	7:06 PM	13.47	0.269	0.170	1.59	1.47	50.00	230	0.183	-0.005
QC0333962-04	Default	2/7/2007	7:07 PM	11.42	0.228	0.124	1.84	1.50	50.00	230	0.153	0.009
QC0333962-04	Default	2/7/2007	7:08 PM	13.34	0.267	0.161	1.66	1.55	50.00	230	0.172	-0.001
Water	Default	2/7/2007	7:11 PM	-1.19	-0.024	-0.004	6.67	0.04	50.00	230	-0.648	-0.008
Water	Default	2/7/2007	7:13 PM	-1.17	-0.023	-0.002	14.61	0.04	50.00	230	-0.654	-0.014

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