



Biopharmaceutical Development Program

Standard Operating Procedure

Title: PicoGreen® dsDNA Quantitation Assay

SOP Number: 22156

Revision Number: 03

Supersedes: Revision 02

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Originator/Date:

Approval/Date:

Approval/Date:

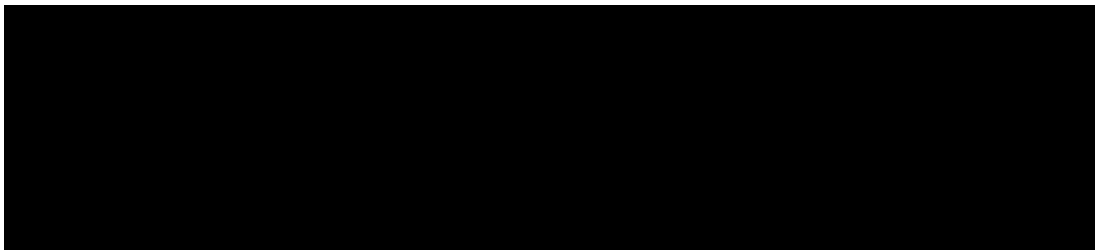


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1.0 Purpose

The PicoGreen® dsDNA assay is used for detection and quantitation of double-stranded DNA (dsDNA) in an aqueous solution.

2.0 Scope

This SOP will be used by Process Analytics (PA) personnel.

3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics 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 operation.

4.0 Materials and Equipment

4.1 PicoGreen® dsDNA Quantitation Kit:

- 4.1.1 PicoGreen® dsDNA Quantitation Reagent (**Component A**), BDP PN 30171 (for Component A only), or BDP PN 30170 (for Components A, B and C), 1.0 mL concentrated dye in high-quality, anhydrous dimethylsulfoxide (DMSO) either in a single vial (Molecular Probes PN P-7589) or in 10 vials of 100 µL each (Molecular Probes PN P-11496). The storage temperature is 2-8°C but may be frozen (-15 to -25°C) for long-term storage.
- 4.1.2 20X TE (**Component B**), 25 mL of 200 mM Tris-HCl, 20 mM EDTA, pH 7.5, certified to be acid-free and DNase-free. The storage temperature is 2-8°C but may be frozen (-15 to -25°C) for long-term storage.
- 4.1.3 Lambda DNA standard (**Component C**), 1.0 mL at 100 µg/mL in TE. The storage temperature is 2-8°C but may be frozen (-15 to -25°C) for long-term storage.
- 4.2 Clean, plastic, 1.8 mL centrifuge, BDP PN 20394 or BDP approved equivalent (e.g., Eppendorf) tubes.

Note: Avoid using glass tubes, as the PicoGreen® Quantitation reagent may adsorb to glass surfaces.

Note: When dealing with proteinaceous samples, the operator needs a minimum of 5 tubes for each test article, standard dilution point, and buffer blank.

- 4.3 Spectrophotometer cuvettes (quartz, or disposable BDP PN 21803, 2.0 mL min. volume.)
- 4.4 Sterile, disposable pipette tips with aerosol barriers (0 - 10 µL; BDP PN 20335, 200 µL; BDP PN 20385, 1000 µL; BDP PN 20769, 25 mL, BDP PN 20054) or BDP approved equivalent.
- 4.5 Sterile conical tubes (15 mL, BDP PN 20006, 50 mL, BDP PN 20140).
- 4.6 Tube racks.
- 4.7 Disposable Nitrile gloves, BDP PN 20764-20767.
- 4.8 Aluminum foil, BDP PN 20012.

- 4.9 Precision Pipettes (P10, P100, P200, P1000, Multi-channel or “Pipet-Man”) or BDP approved equivalent.
- 4.10 Distilled Water, 0.2 µm filtered, Milli-Q, 18 Mega Ohm, or BDP approved equivalent, BDP PN 10189 or 10135.
- 4.11 Phenol, TRIS buffer saturated, BDP PN 10249 or BDP approved equivalent.
- 4.12 m-Cresol, 97%, BDP PN 30699 or BDP approved equivalent.
- 4.13 Chloroform, 99.8%, BDP PN 30257 or BDP approved equivalent.
- 4.14 Isoamyl alcohol (3-Methyl-1-Butanol), 98.5% BDP PN 30698 or BDP approved equivalent.
- 4.15 Anhydrous Ethyl Ether, BDP PN 30697 or BDP approved equivalent.
- 4.16 Phenol: Chloroform: Isoamyl Alcohol, (25:24:1v/v), BDP PN 10212 or BDP approved equivalent.
- 4.17 Vortexer.
- 4.18 “Spin-vac” Centrifuge.
- 4.19 Tabletop Ultracentrifuge.
- 4.20 UV/VIS Spectrophotometer.
- 4.21 Spectrofluorometer (Shimadzu).

5.0 Process Criteria

Note: Prior to sample preparation, it must be determined whether the Test Article is a previously purified “nucleic acid” solution or one that is “proteinaceous,” which would, thus require additional steps to remove this potential contaminant. Regardless of the procedure used, the dilutions used for the Standard Curve should undergo the same treatment as those of the Test Article.

The sample must be in an aqueous solution or water, pH 6- 8.5, and must not contain more than 100 mM NaCl or 25 mM MgCl₂.

- 5.1 If the sample was previously purified nucleic acid, then go to Section 8, dsDNA Standard Curve.
- 5.2 If the sample is proteinaceous then follow **SOP 23113, *Purification of DNA and Viral RNA Using QIAamps Mini Kits***.
- 5.3 In the event that a sample can not be extracted by the Qiagen method, prepare solutions per Steps 6.3 through 6.5 and follow the sequence of events in steps 7.2.1 through 7.2.5 which are summarized in the flow chart below in section 7.2.5.

6.0 Buffer Preparation

Record information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

6.1 Sample & Standard dsDNA Diluent

- 6.1.1 The sample buffer, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE), is used for diluting the PicoGreen® reagent, sample preparation, and subsequent dilution of the DNA samples as well as standard in the assay itself. Because the PicoGreen® dye is an extremely sensitive detection reagent for dsDNA, it is imperative that the TE buffer is free of contaminating nucleic acids.

Note: The 20X TE buffer (Component B) included in the PicoGreen® dsDNA Quantitation Kit is certified to be nucleic acid-free and DNase-free.

- 6.1.2 Prepare the 1X TE working buffer solution by diluting the concentrated buffer (**Component B**) 20-fold with filtered, distilled water. For example, add 1 mL of the 20X TE buffer to 19 mL Gibco BRL Distilled water. (This may be prepared and stored up to two (2) weeks prior to use, and within the expiry date of the 20x TE).
- 6.1.3 If the number of samples require a larger volume of than 20 mL, use appropriate volumes of each ingredient in the same ratio of volumes.

6.2 PicoGreen® Reagent Preparation

Record information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

CAUTION: Because PicoGreen® reagent binds to nucleic acids, it should be treated as a potential mutagen. It should be handled, and disposed of appropriately.

- 6.2.1 On the day of the experiment, allow Component A to warm to room temperature, or in a 37°C ± 2°C water bath for 5-8 minutes before using.
- 6.2.2 In a plastic container, prepare an aqueous working solution of the PicoGreen® reagent by making a 200-fold dilution of the DMSO stock solution (**Component A**) with the 1X TE buffer prepared in step 6.1.2. (Use plastic containers since DMSO may adsorb to glass surfaces.)
- 6.2.3 The PicoGreen® reagent is very susceptible to photodegradation and must be protected from light by covering it with foil or placing it in the dark at room temperature until time of use.
- 6.2.4 For best results, use this solution within a few hours of its preparation.

6.3 Phenol/m-Cresol Solution (249:1)

This solution may be prepared and stored at room temperature up to three (3) days prior to use. Record the information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

- 6.3.1 249.0 mL saturated Phenol, pH 8.0 ± 0.2 .
- 6.3.2 1.0 mL m-Cresol.
- 6.3.3 Mix well and let the aqueous and organic phases separate for > 5 minutes at room temperature.
- 6.3.4 Only use the lower organic phase in subsequent extraction steps.

Note: To ensure that the pH of the saturated Phenol is within the indicated range on the same day as the extraction. If necessary, adjust the pH higher by adding a volume of Tris Base (pH 8.2) equal to the volume of Phenol and re-saturate the solution. No pH reading of the Cresol is needed.

6.4 Chloroform/Isoamyl Alcohol Solution (24:1) (Prepare in a chemical fume Hood).

This solution may be prepared and stored at room temperature for up to three (3) days prior to use. Record information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

- 6.4.1 24.0 mL Chloroform.
- 6.4.2 1.0 mL Isoamyl alcohol.
- 6.4.3 Mix well.

6.5 Water Saturated Ethyl Ether Solution (1:1)

This solution may be prepared and stored at room temperature for up to three (3) days prior to use. Record information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

- 6.5.1 Combine the following reagents in a > 250 mL bottle within a chemical fume hood:
 - 6.5.1.1 100 mL Anhydrous Ethyl Ether.
 - 6.5.1.2 100 mL 18 Mega Ohm water.
- 6.5.2 Mix well and allow the aqueous and organic phases to separate for > 5 minutes at room temperature.
- 6.5.3 Only use the **upper** organic phase in subsequent extraction steps.

7.0 Sample Preparation

7.1 Previously-Purified Nucleic Acid Samples (including Qiagen column extracts)

- 7.1.1 Most nucleic acid extraction methods will provide DNA of sufficient purity to perform PicoGreen® quantitation. These methods include but are not limited to: Qiagen silica columns (**SOP 22932, Plasmid DNA Isolation Using the Qiagen Purification System**) and other resin-based methods, agarose and PAGE gel extracts, CsCl banding, magnetic bead separation, and other hybridization or desorption-based purification methods. The DNA extraction method used (if any),

including reagent expiration, part, and lot number information must be documented on Form 22156-01, unless additional records capturing this information are already required per the extraction SOP used."

7.1.2 Go directly to **Step 8.0 – dsDNA Standard Curve**.

7.2 **Optional Proteinaceous sample extract:** If Qiagen or other silica, gel, bead, cesium, or resin-based DNA extraction methods do not provide sufficient nucleic acid (DNA) purity, the Phenol extraction method described below may be used independently of, or following, other DNA extraction methods. Refer to Steps 5.2 through 5.3. If necessary, refer to the flow chart below which follows Section 7.2.5 and summarizes Steps 7.2.1 through 7.2.5. The following steps may be captured in the comment Sheet of Form 22156-01 or in a current laboratory notebook.

7.2.1 **Phenol/m-Cresol_(249:1) (Ph/m-Cr) Extraction**

7.2.1.1 **100% Ph/m-Cr Extraction**

a) **Initial Extraction**

1. Aliquot 800 µL of each sample into a clean 1.5 mL microcentrifuge tube.
2. Add an equal volume of Ph/m-Cr and secure the lid.
3. Vortex at low speed for a few seconds to initially mix the solution.
4. Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.
5. Centrifuge at maximum speed (14,000 rpm or 20,800 x g) in a tabletop ultracentrifuge for no longer than 3 minutes.
6. With a sterile pipette, remove 750 µL of the aqueous phase (**top layer**) and place in a clean tube. Label each tube appropriately and set aside the tube containing the aqueous layer.

b) **Back-Extraction of the Organic Phase**

1. To each "initial extraction" tube containing ~ 50 µL residual aqueous phase and the organic phase, add 750 µL TE Buffer and secure the lid.
2. Vortex at low speed for a few seconds to initially mix the solution.
3. Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.
4. Centrifuge at maximum speed (14,000 rpm or 20,800 x g) in a tabletop ultracentrifuge for no longer than 3 minutes.

5. With a sterile pipette, remove 750 µL of the aqueous phase (**top layer**) and add to a new appropriately labeled tube. Discard the remaining organic phase in a hazardous liquid container.
6. Continue with the extraction steps listed below and apply to both the initial and back-extraction samples.
7. The purified initial and back-extraction samples will be combined at Step 7.2.5.

7.2.1.2 **80% Phenol / m-Cresol – 20% Chloroform / Isoamyl Alcohol (29:1) (Ch/Iso) Extraction**

- a) To each tube add 600 µL Ph/m-Cr.
- b) To each tube add 150 µL Ch/Iso and secure the lid.
- c) Vortex at low speed for a few seconds to initially mix the solution.
- d) Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.
- e) Centrifuge at maximum speed (14,000 rpm or 20,800 x g) in a tabletop ultracentrifuge for no longer than 3 minutes.
- f) With a sterile pipette, remove 700 µL of the aqueous phase (**top layer**), place in a clean centrifuge tube and label appropriately

7.2.1.3 **50% Phenol/m-Cresol - 50% Chloroform/Isoamyl Alcohol Extraction**

- a) To each tube add 350 µL Ph/m-Cr.
- b) To each tube add 350 µL Chl/Iso and secure the lid.
- c) Vortex at low speed for a few seconds to initially mix the solution.
- d) Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.
- e) Centrifuge at maximum speed (14,000 rpm or 20,800 x g) in a tabletop ultracentrifuge for no longer than 3 minutes.
- f) With a sterile pipette, remove 650 µL of the aqueous phase (**top layer**), place in a clean centrifuge tube and label appropriately.

7.2.2 **100% Chloroform/Isoamyl Alcohol Extraction**

- 7.2.2.1 To each tube add 650 µL Chl/Iso and secure the lid.
- 7.2.2.2 Vortex at low speed for a few seconds to initially mix the solution.
- 7.2.2.3 Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.

7.2.2.4 Centrifuge at maximum speed (14,000 rpm or 20,800 x g) in a tabletop ultracentrifuge for no longer than 3 minutes.

7.2.2.5 With a sterile pipette, remove 600 µL of the aqueous phase (**top layer**), place in a clean centrifuge tube and label appropriately.

7.2.3 **Water Saturated Ether Organic Extraction**

7.2.3.1 To each tube add 1000 mL water saturated ether (Et/H₂O) and secure the lid.

7.2.3.2 Vortex at low speed for a few seconds to initially mix the solution.

7.2.3.3 Continue gentle mixing by placing on a rocker panel for a minimum of 3 minutes.

7.2.3.4 Centrifuge at maximum speed (14,000 rpm = 20,800 x g) in an Eppendorf Tabletop Ultracentrifuge for no longer than 1 minute.

7.2.3.5 With a sterile pipette, remove as much of the organic phase (**top layer**) as possible and hold for proper disposal as a hazardous liquid. **Retain the aqueous bottom layer in the tube.**

7.2.3.6 Repeat steps 7.2.3.1 through 7.2.3.5 twice, for a total of 3 ether extractions.

7.2.4 **Removal of Volatile Organic Residues**

7.2.4.1 Place each tube into a Spin-Vac Centrifuge.

7.2.4.2 Set the Radiant Cover (RC) to the "OFF" position.

7.2.4.3 Set the Timer to "OFF" on the digital display.

Note: CRITICAL SAFETY REQUIREMENT - The Radiant Cover is a high intensity light that if set to any position other than "OFF" could result in an ether explosion! Pre-test the vacuum centrifuge settings prior to adding samples if in doubt of the current settings.

7.2.4.4 Set the vacuum control to "Full Vacuum."

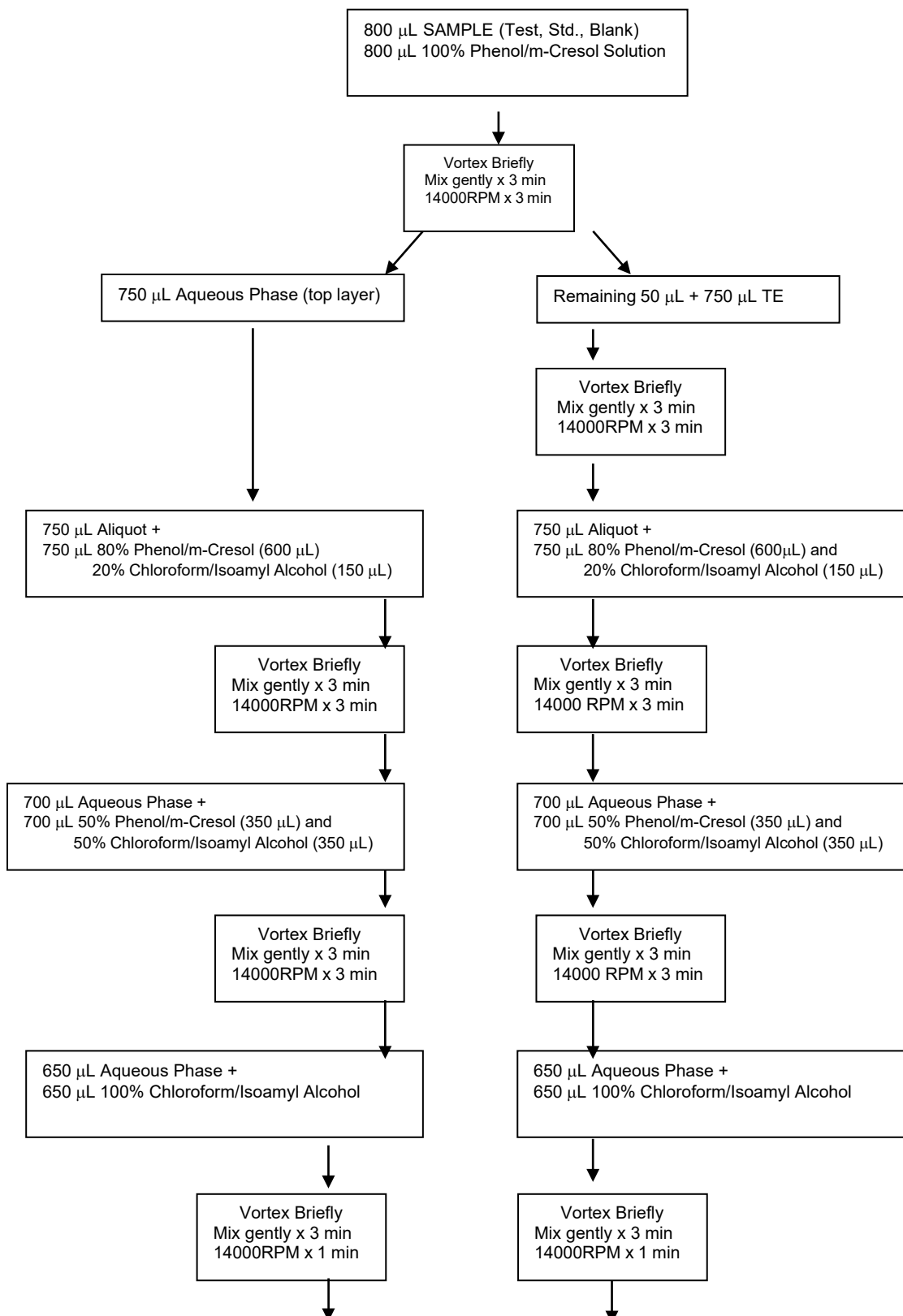
7.2.4.5 Press the manual toggle to the "RUN" position and centrifuge for approximately 1 minute or until all the ether has evaporated.

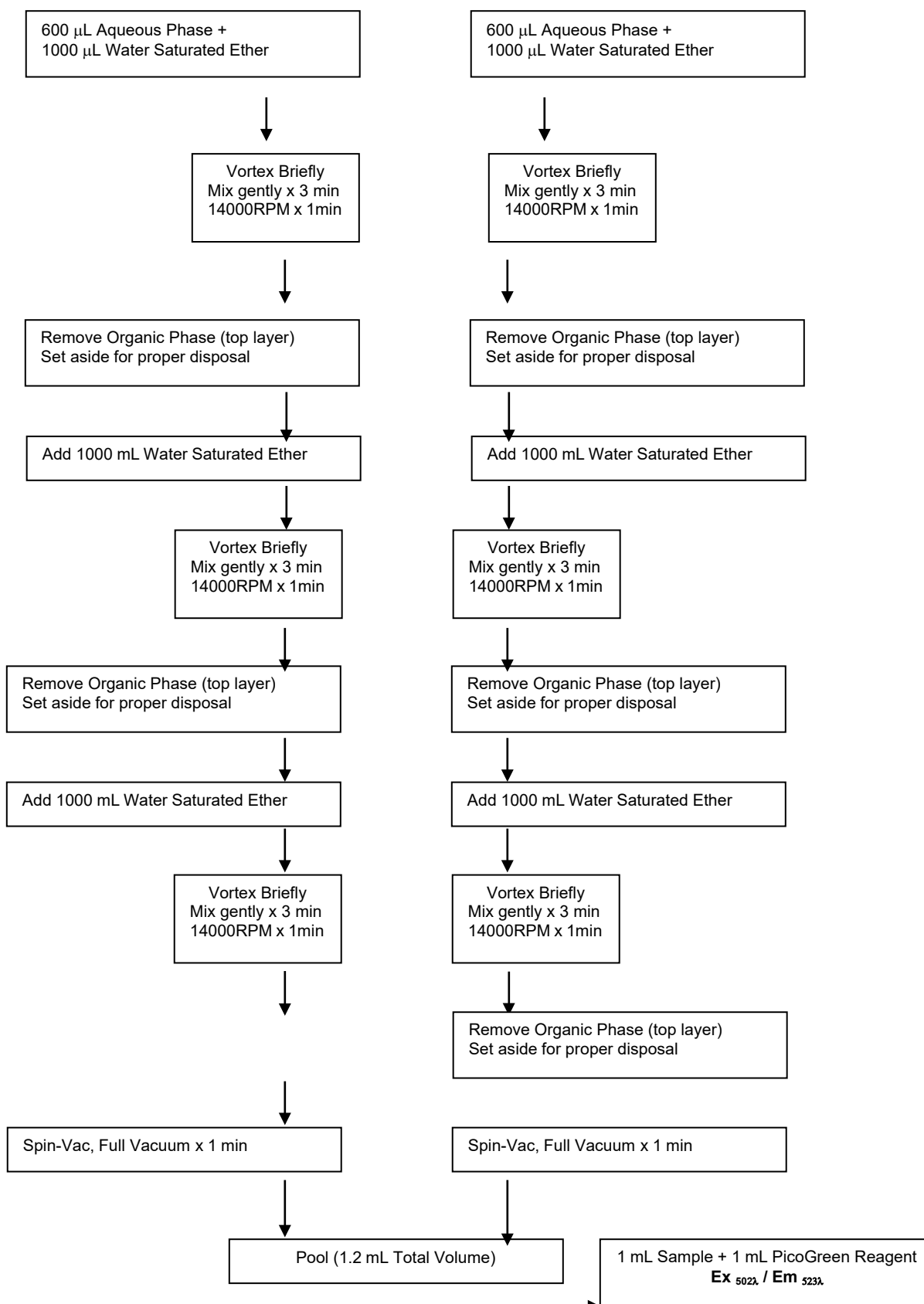
7.2.5 **Pool Sample Replicates**

7.2.5.1 Remove the tubes from the Spin-Vac Centrifuge.

7.2.5.2 Remove 500 µL of each Test Article, Standard and Blank, pool any replicates into clean centrifuge tubes and label accordingly.

Flowchart of Preparation of Proteinaceous Samples





8.0 dsDNA Standard Curve

Refer to the following flowchart.

Note: Prior to Standard Curve preparation, it must be determined whether the Test Article is a previously purified nucleic acid solution or one that is proteinaceous, which would thus, require additional steps to remove this potential contaminant. Regardless of the procedure used, the dilutions used for the Standard Curve should undergo the same treatment and use the same buffer solution as that of the Test Article.

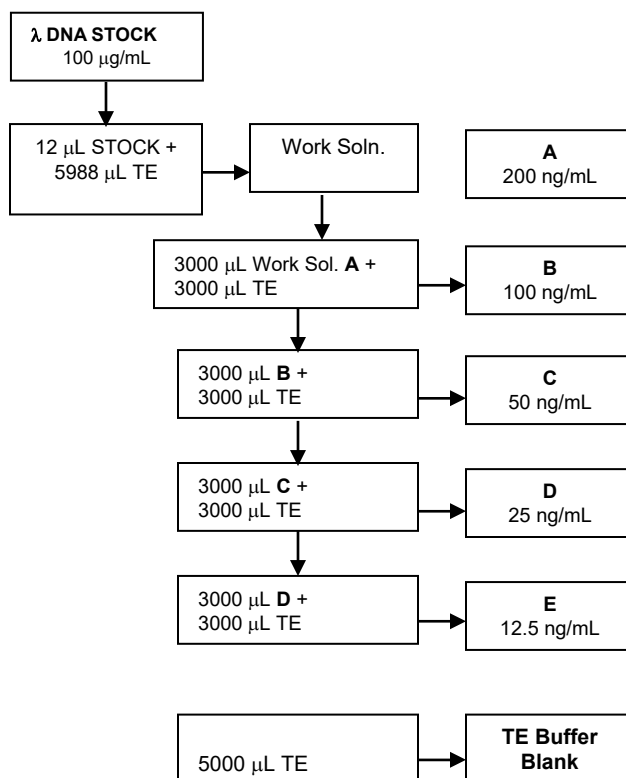
- 8.1 The Lambda DNA Standard (**Component C**) is provided at 100 $\mu\text{L/mL}$ in the dsDNA Quantitation kit. Verify its concentration on the basis of absorbance at 260 nm in 10 mm light path.
- 8.1.1 Use 1X TE buffer prepared in Step 6.1.2 to make a 5x dilution of the lambda DNA standard.
- 8.1.2 By way of a calibrated UV/VIS spectrophotometer, determine A_{260} of the diluted lambda DNA standard. As a reference, it should be noted that λ_{260} value of 0.4 corresponds to dsDNA concentration of 20 $\mu\text{g/mL}$ (5x dilution of **Component C**) in an aqueous solution.
- 8.1.3 Record the determined absorbance value (λ_{260}) on Form 22156-01. Attach the pertinent data print-outs to Form 22156-01
- 8.1.4 Prepare Working Solution and Subsequent Dilutions (A-E) of dsDNA Stock for Standard Curve according to the table below. For example, 12 μL of the λ DNA Stock mixed with 5.988 mL of TE will be sufficient for a six-point standard curve (0 - 100 ng/mL Final). Record information on PicoGreen® dsDNA Quantitation Analysis, Form 22156-01 (Attachment 1).

Flowchart for Standard Curve Production

	μg	Ng	Pg	
Stock	100	100000	100000000	/mL

		Initial (tube)			→	Final (cuvette)			
		μg	Ng	Pg		μg	ng	pg	
A =	Working Solution: 12 μL Stock + 5.988 μL TE	0.2	200	200000	/mL	0.1	100	100000	/mL
B =	3000 μL A + 3000 μL TE	0.1	100	100000	/mL	0.05	50	50000	/mL
C =	3000 μL B + 3000 μL TE	0.05	50	50000	/mL	0.025	25	25000	/mL
D =	3000 μL C + 3000 μL TE	0.025	25	25000	/mL	0.0125	12.5	12500	/mL
E =	3000 μL D + 3000 μL TE	0.0125	12.5	12500	/mL	0.00625	6.25	6250	/mL
Blank =	3000 μL TE	0	0	0	/mL	0	0	0	/mL

The change in the final (cuvette) concentration reflects the addition of 1 mL of prepared PicoGreen® dye solution to the sample (1 mL), performed in step 9.2.2.



9.0 Sample Analysis

9.1 Turn on the Shimadzu RF5301PC Spectrofluorophotometer and ensure that the lamp switch is at the "ON" position.

9.1.1 Ensure the high-sensitivity, mirrored cuvette holder is located in the instrument's loading bay.

9.1.2 Start the RF-5301PC Fluorescence Software on the instrument PC by double-clicking the Shimadzu icon on the desktop.

9.1.3 Ensure that all instrument initialization checks performed by the software are completed successfully before using the instrument.

9.1.3.1 If the checks are not successfully completed, confirm that the instrument is communicating with the computer. From the "CONFIGURE" menu select "INSTRUMENT." Ensure, or place all "Instrument Parameters," in the ON status to facilitate PC communication with the fluorometer, and confirmation that the lamp is on.

9.1.3.2 If the computer is also rebooted, and initialization fails, do not proceed further. Contact the Supervisor.

9.1.4 Wait at least 10 minutes before using the instrument to allow for the high voltage lamp to achieve thermal equilibrium.

9.1.5 Under the "CONFIGURE" option, select "LOAD PARAMETERS" on the Shimadzu RF5301PC spectrofluorometer software.

9.1.6 Highlight the method entitled “*picoqrn*” and press ENTER. This will automatically:

- a) Default to the “data acquisition - unknown” mode, which in turn;
- b) Sets the Excitation and Emission Wavelengths of the Spectrophotometer to 502 nm and 523 nm, respectively.
- c) Sets the Excitation and Emission slit width to 5.0nm and ensure the Emission filter is set to the “open” position.
- d) Sets the Read Time to 4 seconds.
- e) Sets the number of replicate measurements to 3.
- f) Allows for printing of results for manual statistical analysis:
 1. Normalizing against “buffer blanks.”
 2. Linear regression of Standard Curve.
 3. Calculated dsDNA concentrations.

9.2 Standard 2.0 mL Assay

9.2.1 Aliquot 1.0 mL of each Standard, Test Article and Blank into separate cuvettes.

9.2.2 Add 1.0 mL of the PicoGreen® reagent prepared in Step 6.2. Place in the dark and incubate for 2-5 minutes.

9.2.3 Insert the sample blank cuvette into the holder on the platform and close the lid.

9.2.4 Press AUTO ZERO. The operator should hear a distinctive “acknowledgement tone” when completed.

9.2.5 Click on READ three times in succession, pausing 8 -10 seconds between each click. The instrument will assess the intensity of fluorescence each time “Read” is clicked.

9.2.6 Make appropriate sample dilutions where necessary using the prepared 1 X TE buffer as the diluent. Indicate the sample and diluent volumes used on Form 22156-01.

9.2.7 Repeat step 9.2.5 with each sample until all samples are read.

9.3 Once all standards and samples have been read, save the data to H:\BDP_Public\QC Files\Molecular Biology\Reports\Assay Reports\PicoGreen.

9.3.1 Name the file using 8 or less characters and the default extension. Use the BQC test Request number, if applicable, otherwise use the date followed by a sequential character (‘A’, ‘B’, etc.).

For Example: (010102A.QNT)

- 9.4 Print the data file by selecting "**Manipulate/Data Print...**" from the menu. The individual Fluorescence Intensity as well as the mean values for each triplicate sample reading will be printed.
- 9.5 Enter the mean value of each blank, and standard solution on the chart of Form 22156-02 Excel Worksheet (Attachment 2) for determination of Slope, Y-Intercept, and Correlation (**RSQ**) of the Standard Curve
- 9.6 The mean fluorescence value of the reagent blank will be subtracted from that of each Test Article, and Standard solution, accordingly. This data are used to generate a standard curve of fluorescence (Y-axis) versus DNA concentration (X-axis), record the results on Form 22156-02, Attachment 2.
- 9.6.1 The criterion for acceptance for the Standard Curve is a Correlation Value (RSQ) greater than 0.975.
- 9.7 In Form 22156-02, located in H:\BDP_Public\QC Files\Molecular Biology\ Reports\Assay Reports\PicoGreen\Form 22156-02 template1.XLS, enter volume of samples, and diluent used, the mean fluorescence value of each DNA standard solution, test article, and background sample, if appropriate, for the calculation of dsDNA concentration.
- 9.7.1 Calculation of concentration of dsDNA in the Test Articles within the Excel Worksheet, Form 22156-02 is based on rearranging of algebraic formula,
 $Y = MX + B$ to $X = (Y - B) / M$
- Where
- X** = Calculated dsDNA concentration (ng/mL)
M = Slope of the normalized dsDNA Standard Curve
Y = the mean fluorescence value ($Ex_{502\lambda} / Em_{523\lambda}$) of normalized dsDNA Standard Curve
B = Y-Intercept of normalized dsDNA Standard Curve
- 9.8 Form 22156 is a Read-only document. Save the completed Excel Spreadsheet using the QC Request number in the file name such as "Form 22156-02 QC#012345."
- 9.9 Report the analysis results using Form 22156-02 (Attachment 2). Include all print-outs and raw data.

10.0 Documentation

- 10.1 Complete by hand Form 22156-01.
- 10.2 Complete electronically Form 22156-02.
- 10.3 Attach all the following to the QC Test Request Form22002-01.
- 10.3.1 Raw data UV/VIS spectrophotometer.
- 10.3.2 Raw data of fluorometry.
- 10.3.3 Completed Excel Spreadsheet/Standard Curve, Form 22156-02.

10.4 BQA will maintain completed records for as per **SOP 21407, *Records Retention***.

11.0 References and Related Documents

11.1 **SOP 21407** *Records Retention*

11.2 **SOP 22932** *Plasmid DNA Isolation Using the Qiagen Purification System*

11.3 **SOP 23113** *Purification of DNA and Viral RNA Using QIAamps Mini Kits*

12.0 Attachments

12.1 **Attachment 1** Form 22156-01, PicoGreen® dsDNA Quantitation Analysis

12.2 **Attachment 2** Form 22156-02, PicoGreen® dsDNA Content Determination

Attachment 1

NCI-Frederick
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PicoGreen® dsDNA Quantitation Analysis**Section 1****Equipment & Supplies**

Description	MEF #/ID#	Calibration Due Date
Biosafety Cabinet		
Fume Hood		
Centrifuge		
Centrifuge		
Refrigerator		
Water bath		
Spectrofluorometer		
Calibrated Thermometer		
Calibrated Thermometer		
Spectrophotometer		

Attachment 1 (Continued)

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Supplies

Description	BDP PN	Lot Number	Expiry Date
Buffer bottles			
15 mL Tubes			
50 mL Tubes			
5 mL Pipette tips			
1 mL Pipette tips			
_____µL Pipette tips			
_____µL Pipette tips			
_____mL pipette (serological)			
_____mL pipette (serological)			
_____mL pipette (serological)			
Cuvettes			
Microcentrifuge Tubes			

Attachment 1 (Continued)

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QC Test Request Number	Project Number	
Lot Number	Operator's Name	Date Tested

Sample Preparation Method: Indicate below

Phenol/Chloroform Extraction _____

Qiagen QIAamp Mini Kit (SOP 23113) _____

Phenol/Chloroform Reagent Preparations

Section 2		
Phenol / m-Cresol (249:1) Solution		
Made By	Date Made	
Saturated Phenol	Lot Number _____	Expiration _____
	BDP Number _____	Volume used _____
m-Cresol	Lot Number _____	Preparation Date _____
	BDP Number _____	Volume used _____

Chloroform / Isoamyl Alcohol (24:1) Solution		
Made By	Date Made	
Chloroform	Lot Number _____	Expiration _____
	BDP Number _____	Volume used _____
Isoamyl Alcohol	Lot Number _____	Preparation Date _____
	BDP Number _____	Volume used _____

Attachment 1 (Continued)

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Water Saturated Ether (1:1) Solution		
Made By	Date Made	
Chloroform	Lot Number_____	Expiration_____
	BDP Number_____	Volume used_____
Distilled Water, 0.2µm filtered	Lot Number_____	Preparation Date_____
	BDP Number_____	Volume used_____

Comments: _____

Performed By: _____ Date: _____

Attachment 1 (Continued)

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Section 3		
QC Test Request Number	Project Number	
Lot Number	Operator's Name	Date Tested
Section 3 - PicoGreen® dsDNA Quantitation		
PicoGreen® dsDNA Quantitation Buffer Preparations: 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5)		
Made By	Date Made	
PicoGreen® dsDNA Quantitation Kit: Component B (200 mM Tris-HCl, 20 mM EDTA, pH 7.5)	Lot Number _____ BDP Number _____	Expiration _____ Volume used _____ mL
Distilled Water, 0.2 µm filtered	Lot Number _____ BDP Number _____	Expiration _____ Volume used _____ mL
PicoGreen® dsDNA Quantitation Reagent Preparation		
PicoGreen® dsDNA Quantitation Kit: Component A	Lot Number _____ BDP Number _____	Expiration _____ Volume used _____ µL
PicoGreen® dsDNA Assay Buffer (1X TE)	Lot Number: <u>Same as above</u>	Volume used _____ mL

Attachment 1 (Continued)

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PicoGreen® dsDNA Quantitation DNA Standard Working Solution		
Made By	Date Made	
PicoGreen® dsDNA Lambda DNA standard (Component C)	Mfg. Lot Number _____ BDP Number _____	Expiration _____ Concentration _____ µg/mL Volume used _____ µL
PicoGreen® dsDNA Assay Buffer (1X TE)	Lot Numbers: <u>Same as prepared Above</u>	Volume used _____ mL
Final Concentration	_____	
λ_{260} PicoGreen® dsDNA Lambda DNA standard	Dilution Factor: _____	A_{260} Reading: _____

BQC Reviewed By/Date: _____

Attachment 1 (Continued)

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Sample Dilutions

*ID No.	Description	Dilution Volumes (µL)		
		Sample	1X TE Buffer	PicoGreen
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000
				1000

* To correspond with that of spectrofluorometer which is displayed as the ID# on the monitor screen.

Attachment 2

NCI-Frederick
Form No.: 22156-02
SOP No.: 22156
Revision 03:

Excel Worksheet for PicoGreen dsDNA Content Determination

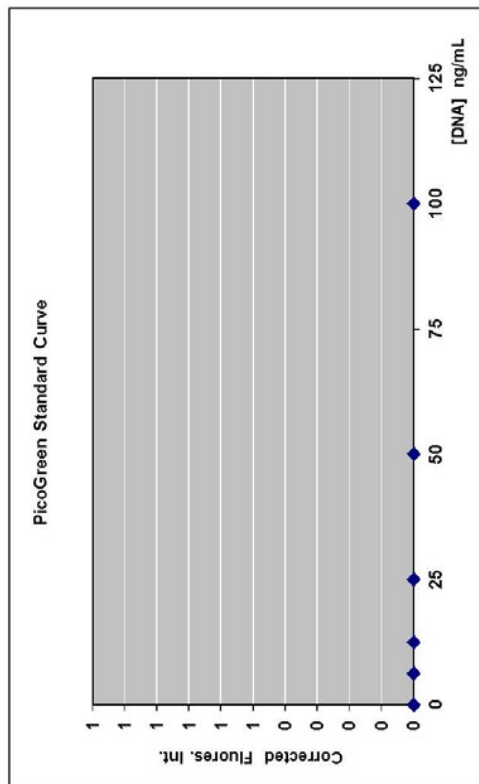
Reviewed By: _____

Date: _____

Project & QC _____

QC Operator: _____

Date: _____



DNA Std.	Fluorescence Intensity	Corrected Intensity
ng/mL		
0		0.000
6.25		0.000
12.5		0.000
25		0.000
50		0.000
100		0.000

Slope 0.0000
Y Intercept 0.0000
RSQ #DIV/0!

Sample No.	Sample Description	Sample Analysis Volumes (µL)			Dilution Factor	Intensity		[dsDNA] ng/mL	Sample Vol. (mL)	DNA per Sample (µg)
		Sample	TE	PicoGreen Reagent		Mean	Corrected			
1				1000	#DIV/0!		0.000	#DIV/0!	1	#DIV/0!
2				1000	#DIV/0!		0.000	#DIV/0!	1	#DIV/0!
3				1000	#DIV/0!		0.000	#DIV/0!	1	#DIV/0!

* If needed, rows may be inserted at the bottom of the table for additional samples.