EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.005_Quantitation with NanoDrop

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 01 October 2007

Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	14 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061520: Review, Renumbered SOP. Previously EIGC.006.

Changes to previous procedures:
041416: Review

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Test Principle: DNA and RNA concentration is determined by reading the absorbance at 260 nm. For the purpose of quality determination, the absorbance is measured at 230, 260, and 280 nm, and the A260/A280 and A260/A230 ratios are determined.

Specimen Type: Isolated DNA or RNA

Safety:

All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Blood borne Pathogen Exposure Control Plan (see Employee Safety Notebook). Gloves must be worn at all times.

Required Reagents:

Nuclease-free distilled de-ionized water or buffer that nucleic acid was isolated in.

Quality Control:

The instrument is calibrated regularly according to the manufacturer's instructions. It is good practice to verify the calibration every six months using CF-1 calibration fluid. Please see calibration records for more information. The samples that fail QC are not to be used in subsequent experiments.

Procedural Steps (general):

1. Place 5 µL of molecular grade water on the pedestal and close the sampling arm. Wipe the pedestal and the arm dry.

2. Open the ND-1000 software and Select 'Nucleic Acids.'

3. Place 1 µL of nuclease-free distilled de-ionized water on the pedestal and close the sampling arm. Initialize the instrument. Wipe the pedestal and the arm dry.

4. Place 1 µL of water or buffer the RNA or DNA was eluted in on the pedestal and close the arm. Select the appropriate workflow (DNA or RNA) from the drop box. Click on 'Blank.' Following completion of the blanking process, wipe the pedestal and the arm dry.

5. Scan, paste, or type the sample name in the sample name box. Place 1 µL of sample onto the pedestal and close the arm. Click on 'Measure.' The results are displayed on the screen once the measurement is complete. Wipe the pedestal and the arm clean.

6. Repeat Step 5 for all samples.

7. Export data tables to appropriate project folder on the PPMS server.
    a. Select “Show Report”
    b. Reports -> Save Report
    c. Select “Export Report Table Only”
    d. Save the report using the project and order numbers for the name to the PPMS folder for the order.

8. Place 5 µL of nuclease-free distilled de-ionized water on the pedestal and close the sampling arm. Wipe the pedestal and the arm dry.

9. Close the ND-1000 software.

Analysis/Interpretation:

260/280 Ratio

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

260/230 Ratio

This ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm.

Benefits/Limitations:

Change in Sample Acidity

Small changes in the pH of the solution will cause the 260/280 to vary. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3.

Wavelength Accuracy of the Spectrophotometers

Although the absorbance of a nucleic acid at 260 nm is generally on a plateau, the absorbance curve at 280 nm is quite steeply sloped.

A slight shift in wavelength accuracy will have a large effect on 260/280 ratios.

For example, a +/- 1 nm shift in wavelength accuracy will result in a +/-0.2 change in the 260/280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the 260/280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

A disadvantage of this method is that single-stranded DNA (ssDNA) and RNA also absorb UV light at 260 nm and can therefore interfere with the results and cause overestimation of the double-stranded DNA (dsDNA) concentration.

Nucleotide Mix in Your Sample

The five nucleotides that comprise DNA and RNA exhibit widely varying 260/280 ratios. The following represent the 260/280 ratios estimated for each nucleotide if measured independently:

Guanine: 1.15

Adenine: 4.50

Cytosine: 1.51

Uracil: 4.00

Thymine: 1.47

The resultant 260:280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the 260/280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA respectively, are "rules of thumb". The actual ratio will depend on the composition of the nucleic acid. Note: RNA will typically have a higher 260/280 ratio due to the higher ratio of Uracil compared to that of Thymine.

The quality and quantity of RNA/DNA are required for proper execution of all downstream experiments. One of the major limitations is that this procedure is not able to distinguish between intact and degraded samples, so additional tests, such as Bioanalyzer analysis (RNA from frozen tissue and cells or DNA), RPL13a assay (RNA from FFPE tissue), traditional agarose gel electrophoresis (DNA) need to be performed.

Troubleshooting:

See user’s manual.