Montreal Neurological Institute and Hospital
McGill University

CBIG-02-007

DNA Quantification
Through NanoDrop Spectrophotometry

Version:	1.0	Supersedes:	NA
Category:	Procedural	Effective date:	01-May-2020

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	01-May-2020

2. SCOPE AND APPLICATION

This protocol is used for quantifying DNA concertation and obtaining the purity ratio through the use of NanoDrop Spectophotometer.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

3.1 Reference to Other C-BIG SOPs or Documents

NA

3.2 Reference to External SOPs or Documents

1. Experimental Therapeutics Program: SOP ETP-P-0003, DNA Extraction from Whole Blood
2. Thermo Scientific Technical Bulletin Rev 2/09, T042-NanoDrop 260/280 and 260/230 ratios

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who wish to quantify extracted whole DNA. All personnel who read this SOP should sign the form found in the “training log” binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
C-BIG	Clinical Biological Imaging and Genetics Repository
ddH_2O	Double Distilled Water
nm	Nanometers
OD	Optical Density
QA	Quality Assurance
QC	Quality Control
RT	Room Temperature
SOP	Standard Operating Procedure
UV	Ultraviolet
µL	Microliter

6. MATERIALS AND EQUIPMENT

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

NOTE: All sample contact materials must be suitable for RNA work (i.e. RNAse-free). Always use clean gloves to prevent inadvertent RNAse contamination during the procedure. All equipment, disposables or reagents can be substituted with equivalent materials following evaluation and approval, unless specified otherwise.

Material/Equipment	Material/Equipment (site specific)
Filtered pipet tips, 0.1-2 µL	
Micropipets, range 2 µL, 1000 µL	
ddH_2O	
Spectrophotometer	NanoDrop Spectrophotometer 2000 (Thermo Scientific, Cat # ND-2000)
DNA Hydratation Solution	DNA Hydratation Solution (Qiagen, Cat # 158916)
Kimwipes	

7. PROCEDURES

Analysis of UV absorption by the nucleotides, provides a simple and accurate estimation of the concentration of nucleic acids in a sample. Purines and pyrimidines in nucleic acid show absorption maxima around 260 nm (e.g., dATP: 259 nm; dCTP: 272 nm; dTTP: 247 nm) should the DNA sample be pure without significant contamination from proteins or organic solvents. The ratio of OD260/OD280 have to be determined to assess the purity of the sample. This method is however limited by the quantity of DNA and the purity of the preparation. Accurate analysis of the DNA preparation may be impeded by the presence of impurities in the sample or if the amount of DNA is too little. In the estimation of total genomic DNA, for example, the presence of RNA, sheared DNA etc. could interfere with the accurate estimation of total high molecular weight genomic DNA.

7.1 Measurement

1. Prepare a 5 µL aliquot of the sample in a tube.
2. Prepare a 5 µL aliquot of DNA Hydration Solution in a tube for the blank.
3. Wash the NanoDrop lower pedestal with 2 µL of ddH_2O and wipe with Kimwipe.
4. Open computer program and select Nucleic Acids option.
5. Blank with 2 µL of DNA Hydration Solution.
6. Place 2 µL of sample on pedestal and lower the arm.
7. Measure the OD by clicking on the measure option on the software.
8. The concentration and the OD260/OD280 ratio are recorded.

NOTE: If measuring more than one sample, make sure to wipe the pedestal with Kimwipe between each sample.

9. When finished, clean the pedestal with Kimwipe and ddH_2O.

7.2 Analysis

1. A ratio between 1.8-2.0 indicates that the absorption in the UV range is due to nucleic acids.
2. A ratio lower than 1.8 indicates the presence of proteins and/or other UV interacting agents.
3. A ratio above 2.0 implies that the samples may be contaminated with chloroform or phenol. In either case (<1.8 or >2.0) it is advisable to re-precipitate the DNA.

8. QUALITY CONTROL / QUALITY ASSURANCE

All the equipment used should be monitored, cleaned and calibrated as by their specific SOPs.

9. APPENDICES/FORMS

9.1 Appendix A – DNA Extraction from Whole Blood (Accelerated Method or Conventional Method)

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