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C-SOP-202: Genomic DNA Purity Measurement using a Nanodrop Spectrophotometer

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

A standard technique for performing purity measurements is UV absorbance with a spectrophotometer. Microvolume spectrophotometers are commonly used for the analysis of nucleic acid samples. They require a small sample volume (0.5–5.0 µl) and are economical, convenient and widely available. Typically, they measure concentration and purity readings for ssDNA, dsDNA and RNA and can provide meaningful insights into the quality of the sample.

Due to some reported limitations with this method, we would recommend deploying Nanodrop UV spectrophotometry ONLY for purity ratio estimations (A260/280 and A260/230) of double-stranded or single-stranded DNA/RNA, especially when destined for downstream applications such as DNA library preparation for whole-genome, amplicon and targeted sequencing.

As an indicator of sample purity, the ratios of the absorbance values at 260 nm vs 280 nm (A260/A280) and at 260 nm vs 230 nm (A260/A230) need to be determined for each sample to ensure its suitability for downstream applications. **The A260/A280** provides insight into the type of nucleic acid present (dsDNA or RNA) as well as an indication of purity. Typically, protein contamination can be detected by a reduction in this ratio; RNA contamination can be detected by an increase in this ratio. In buffered solutions, pure dsDNA has an A260/ A280 of 1.85–1.88.

The **A260/A230** is a sensitive indicator of contaminants that absorb at 230 nm. These contaminants are significantly more numerous than those absorbing at 280 nm, and include chaotropic salts such as guanidine thiocyanate (GTC) and guanidine hydrochloride (GuHCl), EDTA, non-ionic detergents like Triton™ X-100 and Tween® 20, proteins, and phenol. Substances like polysaccharides or free floating solid particles like silica fibers absorb at this wavelength, but will have a weaker effect. In buffered solutions, pure dsDNA has a higher A260/A230 ratios at 2.3–2.4. A260/A230 ratios typically produce a higher standard deviation than A260/ A280 ratios and should be interpreted with care.

This protocol has been adapted from nucleic acid purity measurement assays developed by ThermoFisher Scientific.

PROTOCOL CITATION

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null, Nanodrop, DNA purity, A260/280, Spectrophotometer

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MATERIALS TEXT

1. Genomic DNA

2. Nanodrop 2000/8000 Spectrophotometer

3. Kimberly-Clark™ Professional Kimwipes™ Delicate Task Wipers (Cat no. 33670-04)

4. Nuclease-free Water (Local supplier)

5. Elution buffer stock (used to dissolved the DNA during extraction)

6. Calibrated single- or multichannel pipettes (P2 and P10) with compatible sterile, low-retention, filtered tips.

Optional:
7. 0.1M hydrochloric acid solution

DISCLAIMER:

> This protocol has been adapted from nucleic acid purity measurement assays developed by ThermoFisher Scientific.

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Before Starting 2m

1 1m

An initial cleaning of measurement surfaces with nuclease-free (NF) water is recommended prior to making the blank measurement.

To clean the pedestal, pipette **2 µl** of NF water onto the pedestal and lower the arm. Leave to sit for **00:01:00** and then wipe away with lint-free tissue.
Fig. 1a and 1b illustrate the loading technique and how the droplet sits on the pedestal.

A final cleaning of both measurement surfaces with nuclease-free (NF) water is also recommended after the last sample measurement.

i. **Do not** use a squirt or spray bottle to apply water or any other liquid to the surface of the instrument.

ii. Use 3 µL of 0.1M hydrochloric acid for cleaning if samples have dried on the pedestal. Follow this up with a 3 µL aliquot of NF water.

iii. Detergents and isopropyl alcohol are **not** recommended cleaning agents as they may decondition the pedestal measurement surfaces. If a solution containing detergents or alcohol is used, follow with 5 µL clean of NF water.

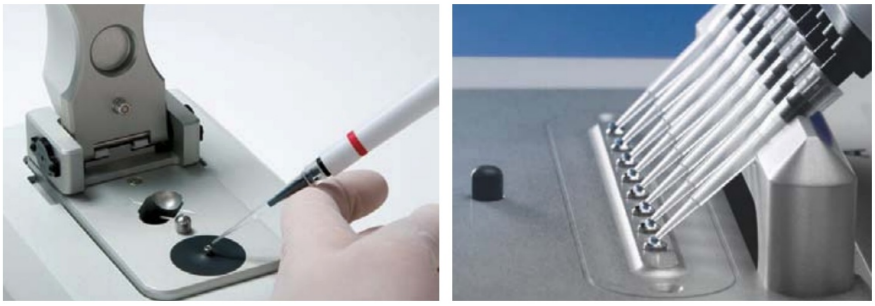

Figure 1a

Figure 1b

2 Prior to initiating the protocol, ensure that all active workbenches are cleaned with 80% ethanol, all relevant personal protective clothing is worn and the work area is prepared for DNA quantification according to local GLP guidelines.

Create an organised bench space by clearing away all clutter in order to maximize work efficiency.

Initialisation

3 Open up the Nanodrop software from the desktop shortcut. Choose the type of sample to be measured (in this case, **Nucleic Acid** , then **dsDNA**)

4

At the prompt, pipette **2 µl** of NF water onto the pedestal and click **OK**. This will complete initialisation of the Nanodrop instrument.

Blank Measurement

5

Once again, select the type of sample that needs to be measured. Load **2 µl** of the **blanking buffer** onto the lower measurement pedestal and lower the sampling arm.

i. The blank solution/buffer should be the same pH and of a similar ionic strength as solution in which the sample has been eluted. For nucleic acid samples, blank buffers is generally low-TE.

ii. Blanking with water for samples dissolved in TE may result in low 260/230 ratios.

i. Use a calibrated pipette with well-fitting tips. It is best to use a precision pipette (P2 or P10) with low retention, precision tips to ensure that sufficient sample is delivered for retention on the pedestal.

ii. Always use fresh tips and fresh aliquots for every measurement.

6 Click **Blank** to measure and store the reference spectrum.

7 After the measurement is complete, use a dry, lint-free lab wipe to remove the buffer from both the top and bottom measurement surfaces (Fig. 2).

Figure 2

8 Pipette another **2 µl** of the blanking buffer onto the pedestal, lower the arm and click **Measure**. The result should be a spectrum that varies no more than 0.04 Abs (10 mm absorbance equivalent) from the baseline at 260 nm. If not, clean the measurement surfaces and repeat steps 1-5.

Sample Measurement

9 After the blank measurement is complete, enter the sample name in the Sample ID box, and choose the appropriate sample type as indicated below in Table 1:

Sample Type	Select Option	Constant Used to Calculate Concentration	Applicable Models
dsDNA	DNA-50	50	All
ssDNA	DNA-33	33	All
RNA	RNA-40	40	All
Oligo	Oligo DNA or Oligo RNA	Oligo Calulator Defined	NanoDrop 2000/2000c and NanoDrop 8000
Oligo	Custom	15 – 150*	NanoDrop 2000/2000c
Oligo	Other	15 – 150*	NanoDrop 8000 and NanoDrop 1000

Table 1

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Pipette **2 µl** of the gDNA sample onto the lower measurement pedestal and lower the sampling arm. Click **Measure**.

i. Use a calibrated pipette with well-fitting tips. It is best to use a precision pipette (0.1-10 µL) with low retention, precision tips to ensure that sufficient sample is delivered for retention on the pedestal.

ii. Always use fresh tips and fresh aliquots for every measurement.

iii. Highly concentrated DNA samples require careful attention to ensure homogeneity before sampling. A sample not fully in solution can lead to non-reproducible results especially when making small volume measurements. Homogenisation can be easily obtained through heating and/or lightly vortexing the samples prior to measurement.

iv. If measuring more than one sample at a time on the NanoDrop 8000, it is important to use a multi-channel pipette to deliver all 8 samples simultaneously.

11 After the measurement is complete, use a dry, lint-free lab wipe to remove the buffer from both the top and bottom measurement surfaces (Fig. 2).

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Review spectral image to assess sample quality (Fig. 3).

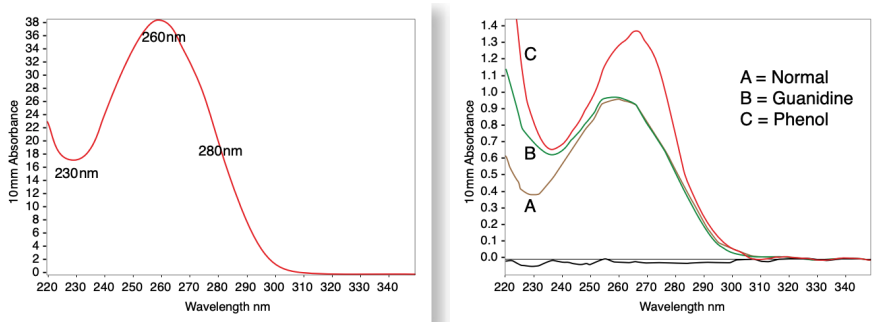

Figure 3. Typical nucleic acid spectrum (left) and comparison of nucleic acid sample spectra with and without 2 common contaminants (right).

Refer to the **Troubleshooting** section on page 17 of the Nanodrop Nucleic Acid Technical Guide for information on aberrant spectral patterns.

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Note down the purity ratios: **A260/280** and **A260/230**.

Refer to the **Purity Ratios** section on page 14 of the Nanodrop Nucleic Acid Technical Guide for information on expected values ranges.

260/280 Ratio:
A 260/280 ratio of **~ 1.8** is generally accepted as "pure" for DNA.
A 260/280 ratio of **~ 2.0** is generally accepted as "pure" for RNA.

The reported 260/280 ratio is dependent on the pH and ionic strength of the buffer used to make the blank measurement and sample measurements. 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.

If the ratio is appreciably different than the accepted target described above, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 260 nm.

260/230 Ratio:
The 260/230 values for a "pure" nucleic acid are often higher than the respective 260/280 values and are commonly in the range of **1.8 – 2.2**.

If the ratio is appreciably different than the accepted target, it may indicate the presence of residual phenol, guanidine, magnetic beads, carbohydrates or proteins.

It is important to ensure that the blank and sample buffers are at the same pH and ionic strength. A low 260/230 ratio may indicate an issue with the buffer used for the blank measurement.

There are typically three common sources of contaminants:
1. Phenol/Trizol extraction — residual reagent contamination may be indicated by abnormal spectra between 220 to 240 nm as well as by shifts in the 260 to 280 nm region.

2. Column extraction — residual guanidine may contribute to a peak near 230 nm and a shift in the trough from 230 nm to ~ 240 nm.

3. Magnetic Beads — residual beads may cause light scatter and result in abnormal spectra. • Samples with concentrations approaching the lower limit of 2 ng/uL may result in unacceptable 260/280 and/or 260/230 ratios.

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Repeat steps 7, 10-12 for each of the next samples.

i. Change to fresh pipette tips before every new measurement.

ii. To manage and import assay readings directly from the instrument, refer to the relevant **Nanodrop User Manual** below.

Additional Information & Troubleshooting

15 Technical Bulletin (ThermoFisher): Interpretation of Nucleic Acid Ratios

Nanodrop Nucleic Acid Technical Guide

Technical Note (New England BioLabs): A practical guide to analysing nucleic acid concentration & purity using MVS

Nanodrop 2000 User Manual

Nanodrop 8000 User Manual

Nanodrop One User Guide