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Title:	**Nanodrop Spectrophotometer**		
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Introduction

Associated Procedure

UV Spectrophotometry

1. Choose the ND-1000 icon from the desktop of the lab computer and select “Nucleic Acid”.

2. Add 5-10 µl of nuclease-free water on the pedestal and close the top to clean the measurement surfaces. Open the sampling arm and wipe the bottom and top pedestal vigorously with a Kimwipe.

3. Use a P2 pipetman and carefully load 2 µl of nuclease-free water into the bottom pedestal of the NanoDrop and gently lower the top pedestal. Click “OK” to initialize.

4. After initialization, select the kind of nucleic acid (DNA, RNA, double stranded, single stranded) you are going to measure.

5. If you want to blank with water (i.e. your nucleic acid has been dissolved in water) then leave the water on the pedestal to blank. Otherwise, wipe the bottom and top pedestal vigorously with a Kimwipe.

6. Load 2 µl of appropriate buffer (that DNA/RNA is dissolved with) onto the bottom pedestal, close the top and click “Blank”.

7. Wipe the bottom and top pedestal vigorously with a Kimwipe.

8. Enter the sample name, etc.. into the Sample ID box.

9. Vortex briefly and quick spin the sample

10. Load from 1-2 ul of sample onto the bottom pedestal of the NanoDrop.

11. Close the top and click “Measure”.

12. Check absorbance ratios and concentration:

13.With wiping the sample from both the upper and lower pedestals upon completion of each sample measurement, it is usually sufficient to prevent sample carryover and avoid residue build-up. Although generally not necessary, 2 ul water aliquots can be used to clean the measurement surfaces after a particularly high concentration sample to ensure no residual sample is retained on either pedestal.

13. **260/280 Ratio:** The 260/280 ratio should be equal to or greater than 1.8 and less than 2.0 in acceptable specimens.

14. **260/230 Ratio:** The 260/230 ratio should be equal to or greater than 1.85 in acceptable specimens (they are commonly in the range of 1.8-2.2).

15. **Concentration:** The concentration range of dsDNA :2 ng/µl to 3700 ng/µl. RNA: 2 ng/µl to 3000 ng/µl; ssDNA: 2 ng/µl to 2400 ng/µl.

16. If you would like to print your report, click “Print Report”.

17. Click “Reports” / “Save Reports”/ “Full Report” and save the report with an appropriate naming convention.

18. If you would like to modify your report click “Show Report” and check that all samples are recorded. Use the “Data” tab to delete all data fields except the parameters of interest (i.e. field showing the final concentration of each sample measured) or to rename samples.

Troubleshooting

- For determination of nucleic acid concentration, dilution of the sample in water is recommended since the relationship between absorbance and concentration (i.e. A260 reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for DNA/RNA in water.
- If DNA/RNA is being extracted for downstream testing and does NOT meet the above acceptable ratio requirements, centrifuge sample using a spin column (i.e. incubate DNA sample on spin column for 1 minute at room temperature. Centrifuge for 1 minute at ≥6000x g (8000rpm) into a new 1.5ml microcentrifuge tube).
- If the concentration of the sample is too high, dilute by adding water or buffer directly to the eluate. Vortex, quick spin and re-measure sample until acceptable range is achieved
- If the concentration of the sample is too low, transfer the eluate back to a spin column and repeat centrifugation for 1 minute at ≥6000 x g (8000rpm). Vortex, quick spin and re-measure sample until acceptable range is achieved.
- Consult with RCEF Coordinator if ratios and concentration are still unattained

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