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Nanodrop

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The Thermo Scientific NanoDrop 8000 Spectrophotometer is a full-spectrum (220-750nm) instrument that accurately measures up to 8 individual 1 ul samples in one measurement cycle. The software allows the user to measure samples using either the full 8-position mode or a convenient single sample mode. The NanoDrop 8000 utilizes the same patented sample retention technology employed on all NanoDrop™ instruments. The surface retention system holds the sample in place eliminating the need for cumbersome cuvettes and other sample containment devices. Clean-up is accomplished in seconds. In addition, the NanoDrop 8000 has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Allyson Hirsch, George Testo 2022. Nanodrop. **protocols.io** https://protocols.io/view/nanodrop-cajdsci6

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Up to eight 1 ul samples are pipetted onto the sample pedestal using a low volume multi-channel pipettor. Each position is actually the end of a fiber optic cable (the receiving fibers). A second set of fiber optic cables (the source fibers) are brought into contact with the liquid samples causing the liquid to bridge the gaps between the fiber optic ends. The pathlengths are automatically controlled to 1mm and 0.2 mm paths. Readings are acquired through sequential measurement across the 8 positions. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light that passes through the samples. The instrument is controlled by PC based software, and the data is logged in an archive file on the PC.

Reagents

- 1 x molecular grade H2O
- 1 x bottle of blanking solution (Buffer EB or RNAase Free H2O or Elution Buffer)

Supplies

- 1 x reagent boat & tubs
- Green temporary seal(s)
- Foil seal(s)

Equipment

- 20uL (traditional) pipette, tip box, & tips
- 10uL 12-channel pipette, tip box, & tips
- 12 well (traditional) Nanodrop

The NanoDrop 8000 is supplied with a 12V power supply. Use only the power supply provided with the instrument. The unit also comes with a grounded power cord. Plug this cord into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply. The power supply can remain plugged into the NanoDrop 8000 while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the flash lamp is not energized. The instrument does not utilize a power switch. It is recommended that the instrument not be positioned in a way that makes it difficult to unplug the power supply from the unit or the wall.

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Preparing & Blanking Nanodrop 10m

10m

1 Allow samples to efficiently thaw before starting (**00:10:00**)

2 Log into computer and open the Nanodrop program and select “Nucleic Acid."

3 Clean Nanodrop pedestals using molecular grade H2O.

Note: Use a Kimwipe to clean and dry sample readers before loading blanks and/or samples.

4 Follow program instructions & load **2 µL** of molecular grade H2O onto each sample pedestal.

5 Blank, then immediately clean sample reader pedestals with molecular grade H2O and a new Kimwipe.

6 Add **2 µL** of blanking solution (solvent used to reconstitute nucleic acids).

Note: The following DNA/RNA extraction kits are associated with these blanking solutions.

- **Qiagen All Prep DNA Kit:** Buffer EB
- **Qiagen All Prep RNA Kit:** RNAse Free Water
- **MagMAX Pathogen RNA/DNA:** Elution Buffer

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7 Blank the machine and clean sample reader pedestals immediately with molecular grade water and a new Kimwipe.

8 Label your samples in the program: can either be pre-programmed or manually entered...

9 Make sure the correct sample desiring to be read is selected: DNA or RNA.

Loading Samples onto Nanodrop

10 Load **2 µL** of sample into the correct sample reader pedestals and click “Measure.”

11 Clean with molecular grade H2O and a new Kimwipe after each run.

12 Repeat steps 10-11 until all samples have been quantified using Nanodrop.

Troubleshooting DNA/RNA Concentrations

12.1 **To determine if samples need a re-run:**

- DNA/RNA concentrations less than 10ng/uL
- A 260/280 ratio outside of 1.8-2.0 for DNA:
- A 260/280 ratio above 2.0 for RNA

Note: If the DNA 260/280 ratio is below 1.8, this may indicate the possibility of contamination or that not enough DNA was extracted!

Cleaning up Sample Pedestals 5m

13 Clean sample reader pedestals after last use with molecular grade H2O and a new Kimwipe; 1m make sure to clean and lower the sample pedestal arm after use.

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14 Save the Nanodrop table information to its correct location. 4m

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