Determining the yield and quality of purified Nucleotides (DNA or RNA) using a Nanovue Spectrophotometer

The Nanovue is a standalone 'cuvetteless' UV/Visible spectrophotometer where a sample of 0.5 - 5 µl may be pipetted directly onto the gold-coloured sample plate for measurement. The sample plate can then be quickly and easily wiped clean with dH_2O for further sample analyses.

Protocol

1. **1. Remove any protective tissue resting between the sampling head and the gold-coloured sampling plate. If necessary wipe the sampling head and plate with clean dH $_2$O and a clean tissue.**

1. **2. Turn the instrument on.**

1. **3. Press '1' for Life Science Folder.**

1. **4. Select application choice. ‘1’ for DNA, ‘2’ for RNA etc.**

1. **5. Review Parameters settings. Pathlength should be ‘Automatic’. Units should be ‘µg/ml’. Dilution factor should be 1.000. Factor should be 50 .0 (DNA) or 40.0 (RNA). Background should be ‘On’. Press ‘OK’ (arrow button).**

1. **6. Take a reference sample*. Avoiding bubbles or spreading of sample, pipette 2 µl of a clean reference sample (i.e. dH $_2$O, Tris-HCL pH 8.0 or TE buffer pH 8.0) onto the sampling plate on the circle at the centre of the cross hairs (Nb. Not the circle at the top of the plate).**

1. **7. Press the reference button (0A/100%T). If the instrument passes calibration, concentration should be displayed as 0.0 µg/ml.**

1. **8. Wipe the sampling head and plate with clean dH_2O and a clean tissue.**

1. **9. Using a clean pipette tip, again avoiding bubbles or spreading of sample, pipette 2 µl of your experimental sample onto the circle at the centre of the cross hairs of the sampling plate.**

10. Press the reference button (half full cuvette with arrow symbol). Concentration of your sample should be displayed as ?.? µg/ml.

11. Take a note of your sample concentration.

12. Wipe the sampling head and plate with clean dH_2O and a clean tissue.

13. If more samples are required, repeat from step 9.

14. If sampling is finished ensure the sampling head and plate are cleaned with dH_2O and a tissue and place a clean/dry folded tissue between the head and plate.

15. Turn the instrument off.

***The default settings for the reference sample should be set to require this to be performed only once.**

- **The Nanovue will display a concentration in µg/ml (converted from the peak absorbance) along with absorbance spectra and ratios for each sample as it is being measured.**

- **DNA should have an absorbance peak centered at a wavelength of 260 nm (A260). The ratio A260/A280 should be between 1.8 -2.0.**

- **The presence of organic solvents (e.g. phenol) may lead to a spuriously high A260/A280 ratio (> 2).**

- **Proteins have a peak absorbance at 280 nm, so protein contamination will lower the A260/A280 ratio. Protein does not absorb as strongly as DNA so even a modest reduction in the A260/A280 ratio (e.g. 10%) for concentrations 4000 ng/µl.**

See also

http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-uk/products/AlternativeProductStructure_16046/#

https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314807262343/litdoc28957475AE_20110831204331.pdf