

Assessment of Nucleic Acid Purity

Introduction

Nucleic acids and proteins have absorbance maxima at 260 and 280 nm, respectively. Historically, the ratio of absorbances at these wavelengths has been used as a measure of purity in both nucleic acid and protein extractions. A ratio of ~ 1.8 is generally accepted as “pure” for DNA; a ratio of ~ 2.0 is generally accepted as “pure” for RNA.

Similarly, absorbance at 230 nm is accepted as being the result of other contamination; therefore the ratio of A_{260}/A_{230} is frequently also calculated. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2.

Residual chemical contamination from nucleic acids extraction procedures may result an overestimation of the nucleic acid concentration and/or negatively influence downstream analysis. Shown below (fig. 1) are example spectra for 4 common extraction reagents which, if not properly cleaned up, will affect sample purity.

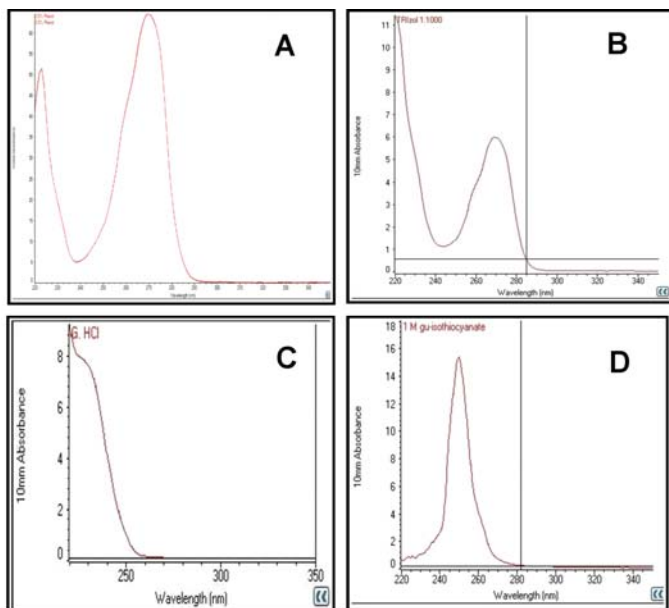


FIGURE 1. Spectra of reagents used in the isolation of nucleic acids. A) TriZol B) Phenol C) Guanidine HCL and D) Guanidinium isocyanate

Contaminant Identification

Examination of sample spectra may be useful in identifying that a problem with sample purity exists. It is recommended that the following be reviewed after each sample measurement:

- 260/230 ratio – a low ratio may be the result of a contaminant absorbing at 230 nm or less.
- 260/280 ratio – a low ratio may be the result of a contaminant absorbing at 280 nm or less.
- Wavelength of the trough in sample spectrum– this should be at ~ 230 nm. Absorbance by a contaminant at a low wavelength will typically shift the wavelength of the trough. Refer to Figure 2.
- Wavelength of the peak in sample spectrum– this should be at 260 nm. Absorbance by a contaminant may shift the peak absorbance wavelength. Refer to Figure 2.

260/230 Ratios

Some contaminants have characteristic profiles, e.g. phenol, however many contaminants present similar characteristics: absorbance at 230 nm or less. Abnormal 260/230 values may indicate a problem with the sample or with the extraction procedure, so it is important to consider both.

A low A_{260}/A_{230} ratio may be the result of:

- Carbohydrate carryover (often a problem with plants).
- Residual phenol from nucleic acid extraction.
- Residual guanidine (often used in column based kits).
- Glycogen used for precipitation.

A high A_{260}/A_{230} ratio may be the result of:

- Making a Blank measurement on a dirty pedestal
- Using an inappropriate solution for the Blank measurement. The blank solution should be the same pH and of a similar ionic strength as the sample solution.

Example: Using water for the Blank measurement for samples dissolved in TE may result in low 260/230 ratios.

260/280 Ratios

Abnormal 260/280 ratios usually indicate that the sample is either contaminated by protein or a reagent such as phenol or that there was an issue with the measurement.

A low A₂₆₀/A₂₈₀ ratio may be caused by:

- Residual phenol or other reagent associated with the extraction protocol
- A very low concentration (> 10 ng/ul).of nucleic acid

High 260/280 purity ratios are not indicative of an issue.

Although purity ratios and spectral profiles are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream application of interest. If the purity ratio is significantly higher than expected, it is best to review the spectral profile as a primary means of troubleshooting.

It is important to note that there are occasions when the purity ratios are within expected limits, yet there is a problem with the sample.

Shifts in Spectral Profile

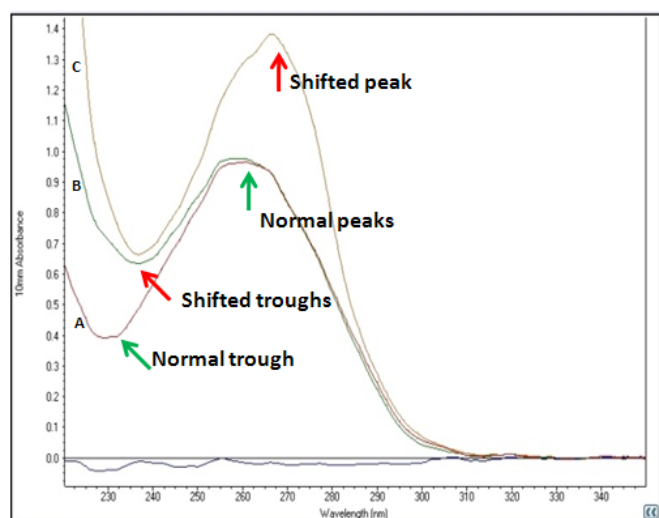


FIGURE 2. Spectra of purified DNA without contamination (A), and of the same DNA sample contaminated with guanidine (B) and phenol (C).

Change in 260/280 Ratios

Some researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to a NanoDrop Spectrophotometer. The two main explanations for this observation are listed below:

- **Change in sample acidity:** Small changes in the pH of the solution will cause the 260/280 to vary (1). 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 comparing results obtained using a NanoDrop Spectrophotometer to results obtained using other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on our instruments is at the same pH and ionic strength as the diluted sample measured on the conventional spectrophotometer.
- **Wavelength Accuracy of the Spectrophotometers** Although the absorbance of a nucleic acid at 260 nm is generally on a plateau, the absorbance curve at 280 nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on 260/280 ratios. It is possible to see as much as a 0.4 difference in the 260/280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within a 1 nm wavelength accuracy specification.

Conclusion

The use of a Thermo Scientific NanoDrop spectrophotometer to QC nucleic acid samples can result in significant savings in time and money. The micro volume capability of NanoDrop™ spectrophotometers allow the researcher to quickly and easily run quality control checks of nucleic acid and protein samples. In addition, the instrument's short measurement cycle and general ease of use greatly increases the rate at which samples can be processed, making it possible to implement multiple quality control checks throughout a procedure or process.

Reference

- 1) William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: *BioTechniques* 22:474-481 (March 1997)