Purity Ratios

Technical Note 130

Introduction

It is common practice for molecular biologists to use the ratio of the measured spectrophotometric absorbance of a sample at 260 nm compared to the value measured at 280 nm as an assessment of purity for nucleic acid and, to a lesser extent, protein samples. Many researchers also look at the ratios of the 230 nm and 260 nm absorbance values as an important secondary measure of purity before using samples in time-consuming or expensive downstream applications.

An example of a nucleic acid sample and a protein sample as measured on a DeNovix® DS-11 spectrophotometer are shown in the figures below:

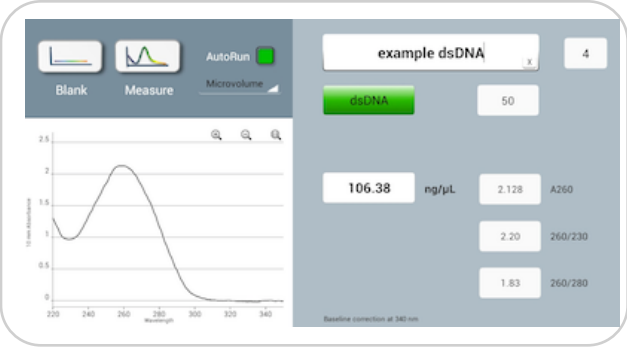

Figure 1: Typical dsDNA spectrum.

Figure 2: Typical BSA spectrum.

260/280 Nucleic Acid Ratios

260/280 ratios are routinely used to determine the purity of nucleic acid measurements. This ratio is most commonly used to determine the presence of protein and/or phenol in the isolated nucleic acid sample. The following chart describes a general acceptable range for these ratios; however they are not a guarantee of sample purity.

Sample Type	Ideal	High	Low
DNA	~ 1.8	>2.0	<1.7
RNA	~ 2.0	>2.2	<1.9
Possible Contamination	-	Basic	Acidic Phenol or Protein

260/230 Nucleic Acid Ratios

The 260/230 ratio is used to indicate the presence of unwanted organic compounds such as Trizol, phenol, Guanidine HCL, and guanidine thiocyanate.

Generally acceptable 260/230 ratios are in the range from 2.0-2.2. Values higher than this may indicate contamination with the aforementioned compounds.

Common Nucleic Acid Contaminants

Despite the best efforts of the researcher, residual contaminants often remain in solution with nucleic acids following chemical isolation. The presence of these can lead to an incorrectly high concentration reading or the disruption of downstream processes.

Protein 260/280 ratio

DNA is a common contaminant of proteins isolated from whole cell lysates. When measuring purified proteins the 260/280 ratio can be a useful tool to determine the purity of your isolated protein. An ideal 260/280 ratio for common proteins is 0.6. Higher ratios may indicate contamination of your isolated proteins with DNA. Alternatively, the buffer used to isolate the sample protein may include components that absorb strongly in the UV region. Buffers and buffer components that fit this description include RIPA buffers, Triton, DTT, urea, and thiourea.

Tip: Colorimetric spectrophotometric methods such as the BCA or Bradford assays are generally recommended when working with buffers with UV region absorbance.

Troubleshooting Purity Ratios

The three most common common causes of abnormal 260/280 ratios are listed below:

- Sample contamination by residual phenol, guanidine, and other reagents used in the isolation protocol. Carbohydrates are a common contaminant associated with nucleic acid isolations from plant sources.
- Samples are very dilute and concentrations are close to the lower detection limit.
    - Inaccurate ratios may be observed for nucleic acids samples with concentrations <10 ng/µl.
- An inappropriate solution was used for the Blank measurement. The Blank solution should b of a similar ionic strength and pH as the sample solution.
    - Using water for the Blank measurement for samples dissolved in TE may result in low 260/230 ratios.

Microvolume vs Cuvette

It has been reported that small changes in the pH of a solution may result in variances in nucleic acid 260/280 ratios[1]. Acidic solutions may under-represent the 260/280 ratio value by 0.2-0.3, while a basic solution may over-represent the ratio value by 0.2-0.3. If comparing ratios for samples measured on the microvolume mode and then diluted to be measured with a cuvette, ensure that the undiluted and diluted sample are at the same ionic strength and pH.

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.)

Variance From Other Instrument Brands

It is not unusual to observe slight differences in purity ratios when measuring the same sample on different spectrophotometers. Up to about a 0.4 difference in the 260/280 ratio may be observed when measuring the same nucleic acid sample on two spectrophotometers that are working within a 1 nm wavelength accuracy specification. The absorbance of nucleic acid at 260 nm is measured within a plateau region of the spectrum, while the 280 nm absorbance is generally measured on a steep sloped portion of the spectral curve. The measurement of one value on a plateau and another on a slope means that a slight shift in wavelength accuracy will have a large effect on 260/280 ratios.

Conclusion

The ratio limits presented previously in this note are generally accepted ideal values. It is important to empirically determine the ratio limits that predict sample functionality in your downstream applications.

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