Tech Note

The Importance of the 240 nm Absorbance Measurement

The A_{260}/A_{280} ratio just isn't enough anymore

The assessment of nucleic acid purity by the A_{260}/A_{280} ratio has been used for a number of years. This measurement, however, does not provide all the information that investigator needs to assess nucleic acid preparations. The detection of impurities common to nucleic acid preparations that are not detected with absorbance measurements at 260 nm and 280 nm can in most instances be accomplished by measuring absorbance at 240 nm. Here we describe the use of the BioTek Instruments PowerWave 200™ scanning microplate spectrophotometer to measure nucleic acid preparations at 240 nm in conjunction with the traditional A_{260}/A_{280} ratio to detect contaminants.

Introduction

The purification of intact nucleic acid from samples is paramount to many applications in molecular biology. Faithful replication of sequences (both RNA and DNA) by PCR in many cases requires nucleic acids that are free of cellular lipids and proteins. Likewise, most restriction endonucleases used to digest genomic DNA are inactivated or degraded by cellular proteases normally present prior to purification of the nucleic acids. Thus, failure to remove these cellular contaminants often leads to poor results.

Removal of the cellular "contaminants" is probably the most basic of all procedures in molecular biology. Removal of cellular proteins is accomplished primarily by the addition of the digestive enzymes such as proteinase K followed by organic extraction with phenol/chloro-form/isoamylalcohol (PCI) mixtures. Cellular membranes are often lysed using detergents such as sodium dodecyl sulfate (SDS). Likewise, many cells contain nucleases that if unchecked may result in degradation of DNA or RNA. In many instances, these nucleases can be inactivated by the addition of divalent ion chelators such as ethylenediaminetetraacetic acid (EDTA).

Materials and Methods

Reagents such as SDS, EDTA, and phenol were molecular biology grade and purchased from Gibco-BRL (Gaithersburg, MD). All solutions including 10% SDS, 0.5 M EDTA and phenol were prepared as described by Maniatis et al (1). Purified genomic herring sperm DNA was digested with EcoRI (Gibco-BRL, Gaithersburg, MD) followed by organic henol/chloroform/isoamylalcohol (PCI) extraction and ethanol precipitation, with subsequent rehydration at 400 µg/ml final concentration. Purified bovine serum albumin (BSA) fraction V, catalogue number A-2153 (Sigma, St. Louis, MO), was dissolved into distilled water at a concentration of 400 µg/ml and filter sterilized.

A series of stock solutions representing various mixtures of DNA and protein were made by mixing differing amounts of the 400 µg/ml DNA and protein stock solutions. To each of these DNA/protein mixes were added SDS, phenol/chloroform/isoamylalcohol (PCI) or EDTA. In each

case, 1% by volume of a stock solution was added. This resulted in final concentrations of 1% PCI, 0.1% SDS, and 5 mM EDTA.

Spectrophotometric measurements were made using a PowerWave 200 scanning microplate spectrophotometer (BioTek Instruments Winooski, VT) in conjunction with Costar (Bedford, MA) UV transparent microplates, catalogue number 3635. Samples were blanked at each wavelength by subtracting from each well the average absorbance of 12 wells containing only water.

Results

The absorbance for a series of protein DNA mixtures was measured at 240 nm, 260 nm, and 280 nm and A260/A280 and A260/A240 ratios were then determined. As demonstrated in Figure 1, DNA/protein mixtures that contain trace amounts of either SDS or EDTA cannot be distinguished from mixtures not containing these common contaminants when A260/A280 ratios are determined. When phenol is present, however, a dramatic change is seen in the A260/A280 ratio.

When the absorbance at 240 nm of these samples is determined EDTA contamination of nucleic acid samples can be distinguished. The ratio of absorbance at 260 nm to that of 240 nm can easily determine the presence of EDTA that could not be determined with the traditional A260/A280 ratio (Figure 2). The presence of PCI can also be distinguished at 240 nm and results in an A260/A240 ratio similar to that seen with the A260/A280 ratio.

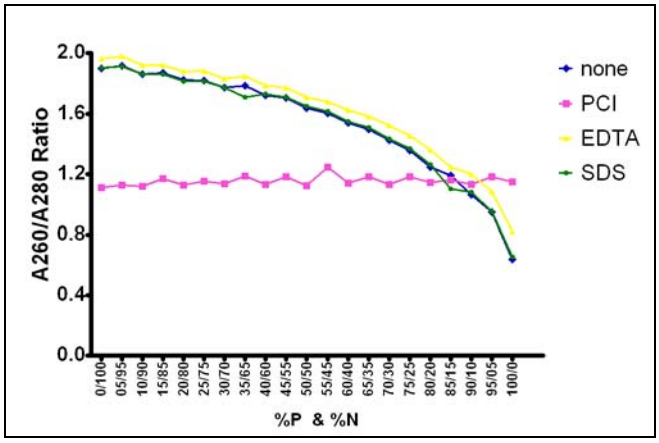

Figure 1. Comparison of A260/A280 ratios of DNA/protein mixtures containing trace amounts of common contaminants of nucleic acid preparations. Protein/DNA mixtures of varying concentrations were prepared as described in the materials and methods section, and 0.1% SDS (5); 5 nM EDTA (s); or 1% PCI (n) were added and compared to samples with no additions (¨). All values represent a mean of eight determinations.

Discussion

Many compounds, including chemicals commonly employed in nucleic acid preparations, absorb light at wavelengths below 260 nm. While these compounds are useful during purification, they may be disastrous if present in later steps. Phenol or mixtures of phenol such as PCI will denature proteins such as restriction endonucleases, polymerases, etc. that may be used in later experiments. EDTA, while not a denaturant, will inhibit reactions that are dependent upon the presence of the divalent cation, Ca++. DNA Polymerases and Dnase I are notable enzymes whose activity is dependent on Ca++ and would be expected to be inhibited by the presence of EDTA in nucleic acid samples.

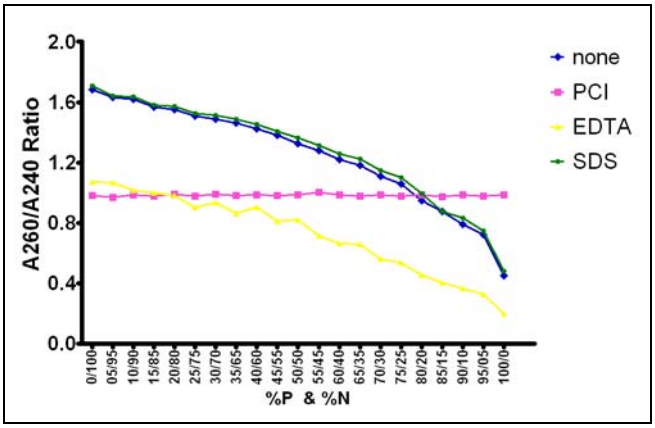

Figure 2. Comp arison of A260/A240 ratios of DNA/protein mixtures containing trace amounts of common contaminants of nucleic acid preparations. Protein/DNA mixtures of varying concentrations were prepared as described in the materials and methods section, and 0.1% SDS (5); 5 mM EDTA (s); or 1% PCI (n) were added and compared to samples with no additions (¨). All values represent a mean of eight determinations.

Although in this report only dsDNA in aqueous solution was tested, other nucleic acids species can be quantitated at 240 nm. Purity of RNA or ssDNA samples, such as oligonucleotides and M13 phage DNA, can be assessed in a similar fashion using UV transparent microplates and the PowerWave 200 scanning microplate spectrophotometer.

In the past such A260/A240 determinations have been performed using the conventional spectrophotometer. This method usually entailed using a pair or at most a set of four matched cuvettes to perform the analysis resulting in a very low throughput. The ability to use the PowerWaveÔ 200 scanning microplate reader to perform this analysis allows this routine procedure to be performed on 96 samples in a matter of seconds leading to a tremendous increase in productivity and throughput.

References

1. Maniatis T., E.F. Fritsch, and J. Sambrook (1982) Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Springs Harbor, NY.

Paul Held, Ph. D.
Senior Scientist & Applications Lab Manager

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