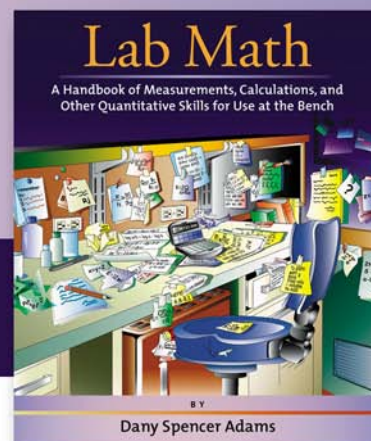


Lab Math

A Handbook of Measurements, Calculations, and
Other Quantitative Skills for Use at the Bench



Errata

from the first printing

Chapter 1

P. 23, in the table, the first line, the Symbol for Unit should read “mol”.

Chapter 5

P. 139–140, **Measuring the concentration of DNA and RNA and Millimolar extinction coefficients** should be replaced with the following:

Measuring the concentration of DNA and RNA

To determine the concentration of DNA or RNA in an uncontaminated solution of nucleic acids using your spectrophotometer, multiply the A_{260} reading by the conversion factors in the table below. These conversion factors are what the concentration (c) would be if A_{260} equaled exactly 1.000; that means they are like unit fractions (see Chapter 1) used to convert A_{260} (which is dimensionless) into a concentration in $\mu\text{g/ml}$ (which is the same as $\text{ng}/\mu\text{l}$). You could say that the units of those conversion factors are μg per ml per “ A_{260} reading” or $[\mu\text{g ml}^{-1} A_{260}^{-1}]$.

You may notice that you are not using the Beer-Lambert law and extinction coefficients for this technique, and you are not getting a concentration in molarity. That is because you can't really do that with nucleic acids. The reason is that the absorbing is being done by nucleotides; the spec cannot distinguish whether those nucleotides are part of a polymer or how long a polymer is. In other words, 2 mM dsDNA that was 6000 bp in length would have the same A_{260} reading as 6 mM dsDNA that was 2000 bp in length; two very different concentrations, but the same A_{260} , because the number of absorbing entities is the same.

Note: This technique cannot distinguish between RNA and DNA. Also, it cannot warn you if your sample is contaminated. Always measure absorbance at a range of wavelengths to test for contamination (see p. 133).

Converting A_{260} to Nucleic Acid Concentration in $\mu\text{g/ml}$

To determine the concentration of an uncontaminated solution of nucleic acids, multiply the value of A_{260} by the appropriate conversion factor.

Nucleic Acid	Conversion Factor*
Single-stranded RNA	40 µg/ml
Single-stranded DNA	33 µg/ml
Double-stranded DNA	50 µg/ml (micrograms per milliliter)

* These conversion factors are appropriate for uncontaminated solutions of nucleic acids over 100 bp in length.

Example

A 2.00-ml sample of ssRNA has an $A_{260} = 0.090$.

The concentration of ssRNA in the sample is: $0.090 \times 40 \text{ µg/ml} = 3.6 \text{ µg/ml}$

If the sample in the cuvette is a dilution of a solution, multiply the concentration of the sample by the dilution factor. For example, if that 2.00 ml sample was a 1:2 dilution of a solution, the solution has a concentration of $3.6 \text{ µg/ml} \times 2 = 7.2 \text{ µg/ml}$

If you need to know the total amount of ssRNA that you have, multiply that concentration times the volume of the solution. Continuing the above example, if you have 5.00 ml of a 7.2 µg/ml solution, you have: $5.00 \text{ ml} \times 7.2 \text{ µg/ml} = 36 \text{ µg}$ of ssRNA

If you know the MW of your nucleic acid, you can determine the concentration (c) in moles per liter using the following conversion:

$$c [\text{µg/ml}] \div \text{MW of nucleic acid [g/mole]} \times 10^{-6} \text{ g/µg} \times 10^3 \text{ ml/l} = c [\text{M}]$$

Chapter 8

P. 238, OTHER UNITS table, the second line, last column (To Convert to SI) should read:

$$\text{kg} = D \div (6.022142 \times 10^{26})$$

For complete information and Table of Contents about *Lab Math*, go to <http://www.cshlpress.com/link/labmath.htm>