

An Introduction to Extinction Coefficients and Molecular Weights of Oligonucleotides

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Every TriLink Certificate of Analysis lists two numbers crucial to the use of an oligonucleotide: the extinction coefficient and the molecular weight. These numbers are needed to calculate important data, such as determining molar concentrations and preparing stock solutions. This paper will offer an explanation of the extinction coefficients and molecular weights of oligonucleotides, discuss the means by which the numbers are derived, and give practical examples on how to use the data provided by TriLink.

The optical density unit, or more commonly the OD₂₆₀ unit, is a spectrophotometric measurement of an oligonucleotide. Each of the bases in a nucleic acid strand has an absorbance at or near 260 nanometers, due to their conjugated double bond systems. Because the exact base sequence and composition is known, the OD₂₆₀ unit is a very accurate and convenient method to quantify an oligonucleotide. The OD₂₆₀ unit is a normalized unit of measurement that is defined as the amount of oligonucleotide required to give an absorbance reading of 1.0 at 260 nanometers in 1.0 milliliter of solution using a 1.0 centimeter light path. Utilizing absorbance measurements is the recommended method for quantitating or aliquoting an oligonucleotide. Often, the total mass of oligonucleotide is too low to accurately weigh on a balance. In addition, spectrophotometric measurements will be accurate regardless of any excess salt present in the sample. An absorbance reading will also ensure that a compound is fully solubilized prior to use.

Associating the OD₂₆₀ unit with the amount of oligo present is done mathematically using a physical constant known as the extinction coefficient. It is a component of Beer's Law: $A = \epsilon Cl$; where A is absorbance, C is concentration, l is the path length, and ϵ is the extinction coefficient, a constant for the material being analyzed. The extinction coefficient takes into account the effects of the neighboring bases as well as the absorbance of each of the individual bases. Because it is dependent on the

exact nucleotide composition and sequence, the extinction coefficient is unique to every oligonucleotide. There are several ways to determine the extinction coefficient of an oligonucleotide. The "nearest neighbor" method gives greater accuracy than the more common method of merely adding the individual bases and multiplying by the individual extinction coefficients. TriLink uses this "nearest neighbor" model to calculate the extinction coefficient of each oligonucleotide. The next page shows the formula for calculating the extinction coefficient as well as the established constants for both the DNA and RNA nucleotides and dimer pairs. TriLink's website conveniently offers a spreadsheet that automatically calculates the extinction coefficient of an oligonucleotide.

The extinction coefficient is listed on TriLink's Certificate of Analysis and is expressed in ODs/ μ mole. These units are mathematically derived and equivalent to the standard equation units, L mmole⁻¹ cm⁻¹. Please note that although TriLink uses the relatively accurate "nearest neighbor" model to calculate the extinction coefficient, it is still just an approximation and may be off by as much as 10%.

Another important piece of data found on TriLink's Certificate of Analysis is the molecular weight. The molecular weight of an oligonucleotide is simply the mass of the compound in grams per mole (6.02 x 10²³ molecules). It is the sum of each of the component molecular weights of all the atoms the oligonucleotide may contain. This value is needed for converting OD₂₆₀ units into units of mass. TriLink gives the molecular weight of the free acid form of the oligonucleotide. The next page shows the information used to calculate the molecular weight of an unmodified oligonucleotide. Since TriLink provides the final yield of the product in OD₂₆₀ units, it is important to understand how to use this data to convert from OD₂₆₀ units into other desired units. Below are examples of conversions to some of the more commonly used units. If you have any further questions, please contact our Client Relations Department. We will be happy to assist you.

Oligo Unit Conversion

Sample sequence:

5' AGC TAA GTC ACT GCC ATT GA 3'

Extinction Coefficient (ϵ):	195.5 OD units/ μ mole
Molecular Weight (MW):	6101.0 g/mole
OD (A ₂₆₀):	32.4

Example 1: Converting to μ moles

$$\begin{aligned} \mu\text{moles of oligo} &= \text{OD (A}_{260}) \div \epsilon \\ &= 32.4 \div 195.5 \text{ OD units}/\mu\text{mole} \\ &= 0.166 \mu\text{moles} \end{aligned}$$

Example 2: Converting to μ grams

(Use the μ moles calculated in Example 1)

$$\begin{aligned} \mu\text{grams of oligo} &= \mu\text{moles} \times \text{MW} \\ &= 0.166 \mu\text{moles} \times 6101.0 \text{ g/mole} \\ &= 1012.8 \mu\text{grams} \end{aligned}$$

*If you are working in L mmole⁻¹cm⁻¹ units, you will first need to convert the ϵ to OD units/ μ mole.

$$\epsilon \text{ L mmole}^{-1}\text{cm}^{-1} \div 1000 = \epsilon \text{ OD units}/\mu\text{mole}$$

$$195,500 \text{ L mmole}^{-1}\text{cm}^{-1} \div 1000 = 195.5 \text{ OD units}/\mu\text{mole}$$