*Modified from IDT Technical Bulletins*

# Everything you ever wanted to know concerning Oligonucleotides but were afraid to ask

## Calculating Oligonucleotide Quantity

### Specifications Sheet Calculations and Conversion Factors

The specific quantity of an oligonucleotide is obtained by the following set of formulas and conversion factors.

The absorbance at 260 nanometers, $OD_{260}$, of each oligonucleotide is read at two different dilutions. The value reported on the specification sheet is the average of these two measurements.

The number of milligrams and nanomoles of the oligonucleotide is calculated from the $OD_{260}$ reading using the following formulas:

For single stranded DNA: 1 $OD_{260}$ = 33 ug = 0.033mg

milligrams = $OD_{260}$ x 0.033

nanomoles = [milligrams ÷ molecular weight] x $10^6$

The molecular weight of each oligonucleotide is presented on that oligo's specification sheet.

## Calculation of Molecular Weight for Synthetic Oligonucleotides

The molecular weight of an oligonucleotide is calculated based on the following formula:

**Molecular Weight (gm/mole)** = (251 x nA) + (245 x nT) + (267 x nG) + (230 x nC)
+ (61 x (n-1)) + (54 x n)+(17 x (n - 1)) + 2

Where:

1) nA = number of dA bases

2) nT = number of T bases

3) nG = number of dG bases

4) nC = number of dC bases

5) n = total number of bases in the sequence

6) (61 x (n - 1)) accounts for the molecular weight of the phosphate groups.

7) (54 x n) accounts for the hydration of the DNA. There are approximately three water molecules per nucleotide.

8) (17 x (n - 1)) accounts for the ammonium cations associated with the phosphate groups.

**Sample Calculation:**

Example sequence:

**ATG TAA TGT TTG GTC CGC**

nA=3, nT=7, nG=5, nC=3, n=18

Using the formula above, the molecular weight of this oligo is 6793 g/mole.

Special modifications such as biotin, 5' phosphate, inosine, uridine, etc. add to the molecular weight of the oligonucleotide.

## Annealing Two Single Stranded Oligos To Make Double Stranded DNA (i.e. How to make linkers)

It is sometimes necessary to make double stranded DNA from single stranded oligos. While the annealing procedure is very simple, attention to a few details can greatly reduce the presence of undesired single stranded material.

**Method:**

1) Dissolve oligos in STE Buffer (10 mM Tris pH 8.0, 50 mM NaCl, 1 mM EDTA). The presence of some salt is necessary for the oligos to hybridize. Dissolve each oligo at high concentration (~10 $OD_{260}$ units / 100 ul).

2) Mix two stands together in equal molar amounts. If you do not there will always be single stranded material left over.

3) Heat to 94°C and gradually cool. For many oligos this can be as simple as transferring to the benchtop at room temperature. For sequences with significant hairpin

potential, a more gradual cooling/annealing step is beneficial; this is easily done by placing the oligos in a water bath or temp block and "unplugging the machine".

4) The resulting product will be in stable, double stranded form and can be stored at 4°C or frozen.

**Things to Consider:**

If the product will be used in a ligation reaction, the addition of 5'-phosphate may be needed. This can be done at the time of oligo synthesis (chemical phosphorylation) or at any time thereafter using PNK (enzymatic phosphorylation).

If the oligos are relatively long or to be used in cloning, starting with PAGE purified oligos is recommended.

## Resuspending Dry Oligos

Oligonucleotides are shipped in dry form. Sometimes the dried DNA becomes dislodged from the bottom of the tube during shipping. This "loose" DNA can easily fly out of the tube when first opened, particularly as electrostatic attraction is present if the user is wearing latex gloves. **ALWAYS BRIEFLY CENTRIFUGE OLIGOS BEFORE OPENING FOR THE FIRST TIME AFTER DELIVERY TO AVOID LOSS OF THE DNA PELLET!**

Pure, dried DNA is usually easy to dissolve in aqueous solution. Not all oligos dry identically and some can take more time to completely resuspend than others. If the oligo solution freezes while lyophilizing in the Speed-Vac the oligo will dry as a filamentous powder that appears like a fine piece of Kleenex or kimwipe. This form of DNA goes almost instantly into solution (but is prone to "fly out of the tube" as noted above). If the DNA does not freeze while drying, it can dry as a clear film coating the bottom and sides of the tube. This form of DNA will also go completely into solution but sometimes requires vigorous vortexing over a period of several minutes to ensure complete resuspension, especially if the oligo yield is large (milligram yields). Heating will speed this process. Phosphorothioate modified oligos are particularly difficult to resuspend. These oligos can be shipped in aqueous solution if requested (they are quite stable).

Dissolve the stock oligo in concentrated form in TE (10 mM Tris pH 8.0, 1 mM EDTA). DNA kept frozen in TE should be stable for years. It is convenient to initially make a freezer stock (which should be thawed relatively infrequently) at 100 uM concentration. Adding a volume of TE (ul) equal to ten times the number of nanomoles of DNA present in the tube (as noted on the spec sheet provided with the oligo) will produce a stock solution at this concentration. For example, dissolve 50 nmoles of oligo in 500 ul TE to make a stock 100 uM solution. Dilute from this stock 1:10 (in water) to make a working solution at 10 uM for use in setting up PCR reactions. Most PCR reactions use

0.1 - 0.5 uM primer. Addition of 1 ul of the 10 uM primer to a 20 ul PCR reaction will result in a final primer concentration of 0.5 uM, or 10 picomoles of oligo in 20 ul volume.

For those preferring to work in mass units, the mass amount of oligo present in each tube is also indicated on the spec sheet, given as both OD260 units and milligrams DNA (1 OD260 = 33 ug single stranded DNA). An average 20-mer primer will have a molecular weight of ~7500, so 1 OD = 33 ug = ~4.4 nmoles. Dissolving 500 ug of DNA in 500 ul TE will result in a stock primer of 1 ug/ul, or about 133 picomoles of DNA. Again, a stock solution at this concentration should be diluted further before use bearing in mind that about 10 picomoles of DNA (or about 75 ng) is sufficient for a single 20 ul PCR reaction.

## Quantitation of Oligonucleotides

### UV Spectrophotometry:

The most accurate method to quantitate mass amounts of nucleic acid is through absorbence spectrophotometry. All samples should be assayed in duplicate. The mass amount of DNA is calculated from the average of these two measurements, assuming 1 $OD_{260}$ = 33 mg for single stranded DNA.

Unincorporated nucleotides and protecting groups can be present in a sample as undesired byproducts from the synthesis can lead to inaccurate estimates of DNA mass. Samples should be purified first by desalting and butanol extraction. After quantitation, each sample should be lyophilized.

### Alternative Methods:

Estimates of DNA mass/concentration can also be obtained from samples run on acrylamide gels. Best results will be obtained using denaturing urea-based gels (as are used in sequencing). DNA bands can reliably be assessed by back-shadowing against a TLC plate using a hand held UV light source (as opposed to transmission fluorescence). The TLC plate will "glow" under UV stimulation; UV light absorbed by DNA bands in the gel will decrease the background fluorescence of the TLC plate and are seen as dark shadows against a light background.

Estimates of DNA mass/concentration cannot be made for single stranded oligonucleotides using ethidium staining with transmission fluorescence/excitation, as is commonly used with longer double-stranded DNA samples. Ethidium binds nucleic acid by intercalation between strands in regions of double-stranded structure. Single-stranded DNA can be detected by ethidium staining, but mass estimation is unreliable this way. Sequence variation leads to the variable introduction of hairpin double-stranded regions in otherwise single-stranded DNA and can lead to wide differences in ethidium staining

intensity. Samples with identical mass/concentration but which differ in sequence can appear very different when visualized on a gel in this fashion. Agarose gels cannot be reliably used to quantitate short, single-stranded oligonucleotides.