Technical Information

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos,
Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers,
Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates
Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Base Composition, A_{260}/ A_{280} Ratio, Purity & Yield

For research use only. Not for use in diagnostic procedures for clinical purposes.

Quantitation of Oligonucleotides by UV Absorbance

Oligonucleotides are most accurately and conveniently quantitated by the measured absorbance of UV light of the sample in a spectrophotometer. Serial dilutions are usually prepared for oligo stock solutions and then a dilution factor used to accurately convert and quantitate the total yield in nmols or micromoles.

According to Beer's law: $A = \varepsilon Cl$:

A = absorbance;

ε = molar extinction coefficient;

C = concentration (mol/L);

l = path length (cm), typically 1 cm.

EC units [liter/(mol.cm)].

The conditions are defined at a specific wavelength, temperature and media, all of which influence "ε". The purine and pyrimidine bases of DNA and RNA strongly absorb light with maxima near 260 nm. A useful approximation is ε = 10,000 for each of the four bases. The bicyclic purines, deoxyadenosine and deoxyguanosine, absorb more strongly (higher extinction coefficients) than the monocyclic pyrimidines, deoxycytidine and thymidine. Using this and other approximations, absorbance can be translated to mass and concentration of oligonucleotides.

An A_{260} (OD) unit is the absorbance of a 1-mL solution, typically in water, measured at 260 nm in a 1-cm path-length cuvette. One unit represents approximately 33 µg of single-stranded oligodeoxynucleotide (DNA). For example, 1 mg of an oligonucleotide is about 30 A_{260} (OD) unit.

At Gene Link all oligo yield is measured by actual A_{260} reading on a spectrophotometer (commonly termed as OD reading at 260 nm) of an aliquot of the synthesized oligo. This reading is then entered into our custom software that calculates the molecular weight of the oligo based on exact base composition and the molecular weight of any modification(s). The extinction coefficient of this specific oligo is then used to calculate the total nmols, total µg, µg/A_{260} and nmol/A_{260}.

The correct yield parameter is the total nmol quantity and NOT the total yield in A_{260} (OD) units or µg. The total yield if measured in A_{260} (OD) units or µg will be erroneous as the same amount of OD and µg will yield varying amounts of nmol for the same size oligo with different base composition. **Gene Link strongly recommends using nmol as the correct measure of quantity and similarly for applications in experimental protocols rather than by weight in µg**. See table below for examples of a 20mer random base composition oligo and 20mer homopolymers.

Similarly A_{260} /A_{280} ratio measurement is not an accurate measure of custom chemically synthesized oligonucleotide quality. A_{260} /A_{280} ratio are used in a molecular biology laboratory where DNA and/or RNA is extracted from cells as an indicator of purity from protein contamination The A_{260} /A_{280} ratio of an oligo varies with base composition even of a 100% (no protein contamination) pure DNA or RNA oligo. Listed below are A 260 / A 280 ratios for crude 20mer oligonucleotides of differing base compositions. See table below for examples.

General Considerations

1. Purine rich oligos (A and G) have higher EC values and thus higher A_{260} thus the nmol/A_{260} will be lower as compared to the same length of C &T homopolymer oligos.
2. Reconstitution of oligos should thus be based on EC based calculation from actual A_{260} of total nmol, total µg, µg/A_{260} and nmol/A_{260}.
3. Gene Link oligo report specifications include total A_{260}, total nmol, total µg, µg/A_{260} and nmol/A_{260}.
4. For critical applications we recommend that the investigator dissolves the oligo in a known volume and perform their own recording of A_{260} to calculate the nmols and other data. This recommendation is to slight laboratory to laboratory variation of spectrophotometric readings.
5. Gene Link does not measure A_{260} /A_{280} ratios as the oligos are synthesized using pure standard bases without any protein or amino acid ever coming into contact. In addition A_{260} /A_{280} ratios to indicate purity will be erroneous as there is variation of A_{260} /A_{280} ratios between oligos of different base composition.

Oligo Base Composition, A_{260}/ A_{280} Ratio, Purity & Yield									
	Base %	%GC	MW	EC(mM* Cm)	µg/A260	nmol/A260	A260/A280 ratio	Lambda Max of Nucleotide	Electrophoretic Mobility
Hetero 20mer	25% of each	50	6,117	188.90	32.90 (33)	5.29	1.66	260 nm	
Oligo dA(20)	100% A	0	6,202	243.40	25.28	4.11	2.50	259 nm	2
Oligo dC(20)	100% C	100	5,721	144.20	39.68	6.93	1.15	280 nm	1
Oligo dG(20)	100% G	100	6,522	203.40	32.07	4.92	1.85	253 nm	4
Oligo dT(20)	100% T	0	6,022	162.60	37.04	6.15	1.14	267 nm	3

Oligo Scale of Synthesis and Typical Yield									
	Crude Desalted			RPC Purified**			Gel Purified		
	20 mer oligo Typical Yield			30 mer oligo Typical Yield			50 mer oligo Typical Yield		
Scale	**A_{260} Units**	**nmols**	**mg**	**A_{260} Units**	**nmols**	**mg**	**A_{260} Units**	**nmols**	**mg**
50 nmol	8-10	30+	0.2-0.3	4-5	12+	0.1-0.16	NR* [1-2]	NR* [2-4]	NR* [0.03-0.06]
200 nmol	20-25	80+	0.6-0.8	8-12	24+	0.26-0.4	4-6	8+	0.13-0.2
1 µmol	100-120	400+	3-4	40-50	90+	1.3-1.6	20-25	40+	0.6-0.8
Purity & Yield	**Purity is greater than 80%** depending on oligo sequence and structure. Refer to coupling efficiency table for oligo length dependent purity and yield. No further purification required for PCR and sequencing applications. Gel purification recommended for oligos above 50 mer and all applications involving cloning and mutagenesis. **RPC is reverse phase purification using a cartridge; a substitute for HPLC.			**Purity 85% to 95%** depending on oligo sequence and structure Yield and Purity will be lower for sequences with high GC content Not recommended for oligos longer than 35 mer. **RPC is reverse phase purification using a cartridge; a substitute for HPLC.			**Purity 98% to ~100%** depending on oligo sequence and structure Yield will gradually decrease as length of oligo increases. Palindromes, hairpins and high GC content oligos and oligos containing stretches of 3 or more G's induce strong secondary structure and base stacking thus decreasing purity and yield. *NR Not Recommended		