

Quantification of Oligonucleotides

The quantity of an oligonucleotide usually is given as its total optical density (OD value), as amount of substance (nmol) or as its available mass (μg). The OD value is measured experimentally, then the other values are calculated.

OD Value

The OD value of a sample at a wavelength of 260 nm is defined as the extinction that occurs in measuring absorption of the sample in 1 ml aqueous solution in a 1 cm cuvette at the respective wavelength.

In practice, OD values are often outside of the linear measurement range of an UV spectrometer; therefore diluted samples have to be quantified and to be extrapolated to the total OD value.

Example:

The dried oligonucleotide is dissolved in 400 μl of sterile water. From this stock solution an aliquot of 10 μl is taken and filled up with sterile water to 1 ml. Now the extinction value of this diluted solution is measured in a photometer in a 1 cm quartz cuvette at a wavelength of 260 nm. The obtained extinction (e.g. 0,25) is multiplied with the aliquotation factor (400 μl / 10 μl = 40). The result is the OD value (10 in our example).

Amount of oligonucleotide in nmol

According to the law of Lambert and Beer ($E = \text{Epsilon} * C * d$) one can convert from the extinction E (OD value) to the concentration C and therefore to the amount of substance of the oligonucleotide. Strictly speaking, the extinction coefficient Epsilon is different for each oligonucleotide sequence and would have to be ascertained empirically. Using so-called nearest-neighbour methods the extinction coefficient of an oligo can also be obtained computationally with acceptable precision. In a good approximation it corresponds to the sum of the extinction coefficients of the individual nucleotides in the sequence. Using the oligo's sequence data and the OD value one can calculate the amount of material as follows:

$$n[\text{nmol}] = \frac{100 \times n[\text{OD}]}{1,54 \times A + 1,17 \times G + 0,75 \times C + 0,92 \times T}$$

n [OD]: OD value

n [nmol]: Amount in nmol

A,G,C,T: Number of the respective bases in the oligonucleotide

For mixed sequences the following values for the amount of material (nmol) result depending on oligo length:

OD value	Oligo length [Number of nucleotides]									
	10	20	30	40	50	60	70	80	90	100
1	9,4	4,7	3,1	2,3	1,9	1,6	1,3	1,2	1,0	0,9
3	28	14	9	7	5,6	4,7	4,0	3,5	3,1	2,8
10	94	47	31	23	19	16	13	12	10	9
25	234	117	78	59	47	39	33	29	26	23
100	937	468	312	234	187	156	134	117	104	94

Thus, for a rough estimate applies for a mixed 20mer oligonucleotide :

1 OD approximates 5 nmol (5000 pmol).

Amount of oligonucleotide in µg

Using the amount of substance in nmol and the oligo's molecular weight, one can calculate the amount of oligonucleotide in µg:

$$n[\mu g] = \frac{n[nmol] \times MW[g/mol]}{1000}$$

n [µg]: Amount in µg

n [nmol]: Amount in nmol

MW: Molecular weight

Also, for rough estimates applies:

1 OD approximates 30 µg DNA oligonucleotide

Calculating concentrations

1. Concentration adjustment to x pmol/µl

A dried oligonucleotide is to be dissolved to obtain a concentration of x pmol/µl.

$$v[\mu l] = \frac{1000 \times n[nmol]}{c[pmol/\mu l]}$$

n [nmol]: Amount in nmol

c [pmol/µl]: Concentration

v [µl]: Volume of the oligo

Example:

An oligonucleotide (54 nmol) is to be dissolved with a target concentration of 50 pmol/µl in the resulting solution:

$$v[\mu l] = 1000 * 54 \text{ nmol} / 50 \text{ pmol}/\mu l = 1080 \mu l$$

Remark:

The following concentrations are identical:

$$c[pmol/\mu l] = c[nmol/ml] = c[\mu mol/l] = c[\mu M]$$

Example:

100 pmol/μl = 100 nmol/ml = 100 μmol/l = 100 μM

2. Concentration when dissolving in a defined volume:

A dried oligonucleotide is to be dissolved in a defined volume of water. Which concentration results ?

$$c[\text{pmol}/\mu\text{l}] = \frac{1000 \times n[\text{nmol}]}{v[\mu\text{l}]}$$

n [nmol]: Amount of substance in nmol

c [pmol/μl]: Concentration

v [μl]: Volumen of the oligos

Example:

An oligonucleotide (54 nmol) is to be dissolved in 200 μl water. Which concentration will result ?

$c[\text{pmol}/\mu\text{l}] = 1000 * 54 \text{ nmol} / 200 \mu\text{l} = 270 \text{ pmol}/\mu\text{l}$

Calculation of the molecular weight

The molecular weight of an oligonucleotide is calculated from the number of individual nucleotides in the oligonucleotide and from possible modifications of the oligonucleotide:

$$\text{MW}_{\text{oligo}} = 313,2 * A + 329,2 * G + 289,2 * C + 304,2 * T + \text{MW}_{\text{mod}} - 61 * [\text{g/mol}]$$

A,G,C,T: Number of bases in the oligo

MW_{mod}: Molecular weight of a modification, if present

Example:

5'- CCA GGC AGT CTT ATT TTG ACT-3'

$\text{MW} = 313,2 * 4 + 329,2 * 4 + 289,2 * 5 + 304,2 * 8 - 61 = 6388,2 \text{ g/mol}$

Melting temperature T_m

The melting temperature T_m of a DNA double strand is defined as the temperature at which 50% of the double strand have been denaturated, thus being present in single stranded form. Length of the oligo, base composition and concentration of an oligonucleotide as well as the salt concentration in the solution have a key influences on the T_m value. There are different more or less exact methods of predicting the melting temperature:

a) Wallace Rule (2 + 4 Rule) [1]

This rule which is valid for very short oligonucleotides (up to about 15 bases) assumes a contribution of 2 °C for each AT-pair and of 4°C for each GC-pair to the melting temperature of a double strand:

$$T_m = 2^\circ\text{C} \times (A + T) + 4^\circ\text{C} \times (G + C)$$

The rule was established for hybridizations to membrane-bound oligonucleotides and assumes a salt concentration of 1 M. For solution-based experiments one should add 8°C to the calculated temperature.

b) Calculation by GC-content

The following formula based on Howly et al. [2][3] takes mostly into account the GC content of the oligo and is valid for long oligonucleotides:

$$T_m = 81,5 + 0,41 (\%GC) + 16,6 \log c(M^+) - 500/n - 0,61 (\%F) - 1,2 D$$

with

- %GC = Percentage of GC-pairs
- c(M⁺) = Concentration of mono-valent cations
- n = Number of nucleotides
- %F = Percentage of formamide in the buffer
- D = Percentage of mismatches

c) Nearest-Neighbor Method

The so-called Nearest-Neighbor-Method also takes into account sequence-dependent stacking effects for T_m calculation and is based on thermodynamic properties of neighbouring nucleotide pairs. This method is reliable for oligonucleotides of medium length (20 – 60 bases).

Formula [4]:

$$T_m = [(1000 \times dH) / (A + dS + R \times \ln (C/4))] - 273,15 + 16,6 \times \log c(K^+)$$

with

- dH = Sum of the enthalpies of all pairs
- dS = Sum of the entropies of all pairs
- A = -10.8 cal, Entropy of helix formation
- R = 1.984 cal/grad x mol, Gas constant
- C = Oligonucleotide concentration (250 pmol/l)
- c(K⁺) = Concentration of potassic ions in the oligo solution (50 mmol/l)

$$T_m = \left(\frac{1000 \times dH}{A + dS + R \times \ln \left(\frac{C}{4} \right)} \right) - 273,15 + 16,6 \times \log_c (K^+)$$

Thermodynamic data [5][6]:

Dinucleotide	Enthalpy dH (kcal)	Entropy dS (cal)
AA	-9.1	-24.0
AG	-7.8	-20.8
AC	-6.5	-17.3
AT	-8.6	-23.9
GA	-5.6	-13.5
GG	-11.0	-26.6
GC	-11.1	-26.7
GT	-6.5	-17.3
CA	-5.8	-12.9
CG	-11.9	-27.8
CC	-11.0	-26.6
CT	-7.8	-20.8
TA	-6.0	-16.9
TG	-5.8	-12.9
TC	-5.6	-13.5
TT	-9.1	-24.0

Example:

Oligo: 5'-GTC GAA CCG GAA ACC ACC CCT-3'

$dH = -6.5 -5.6 -11.9 -5.6 -9.1 -6.5 -11.0 -11.9 -11.0 -5.6 -9.1 -9.1 -6.5 -11.0 -5.8 -6.5 -11.0 -11.0 -11.0 -7.8 = -173.5$

$dS = -17.3 -13.5 -27.8 -13.5 -24.0 -17.3 -26.6 -27.8 -26.6 -13.5 -24.0 -24.0 -17.3 -26.6 -12.9 -17.3 -26.6 -26.6 -26.6 -20.8 = -430.6$

$T_m = (1000 \times (-173.5)) / ((-10.8 -430.6 + 1.984 \times \ln(6.25 \times 10^{-11})) - 273.15 + 16.6 \log 50 \times 10^{-3}) = 60.7$

All methods for the calculation of melting temperatures given above do not take into account modifications of the oligonucleotide (e.g. dyes, linkers).

Literature

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