Purification abbreviation

Crude	Precipitated
FPLC	Fast Protein Liquid Chromatography
G25	Column Desalting
HPLC-IEX	Ion-Exchange HPLC
HPLC-RP	Reverse Phase HPLC
PAGE	PolyAcrylamide Gel Electrophoresis
RP-Cart	Reverse Phase Cartridge
RP-LP	Reverse Phase Cartridge Long Process
SePOP	Selective Precipitation Optimized Process
UltraF	UltraFiltration

QC abbreviation

CGE	Capillary Gel Electrophoresis
ESI	ElectroSpray Ionisation Mass Spectrometry
FLUO	Fluorescence measurement
IEX	Ion-Exchange HPLC
MS	Maldi-TOF Mass Spectrometry
RP	Reverse Phase-HPLC
SEQ	MS Sequencing
UPLC	Ultra Performance Liquid Chromatography

Reconstitution

- Spin the tube briefly to collect the DNA in the bottom of the tube. Add an appropriate volume of sterile TE buffer (10 mM Tris-HCl, 0.1-1 mM EDTA ; pH 7.5-8.0) or dH_2O. Allow the tube to stand for a few minutes at room temperature, then vortex it for 15 seconds. Please note that some oligonucleotides (i.e. milligram amounts or phosphorothioate oligonucleotides) are particularly difficult to resuspend and may require longer incubation times and/or thorough vortexing. Heating may also help to speed up the process.
- For optimal long-term storage of fluorescent dye-labeled oligonucleotides, it is recommended that the oligonucleotides be resuspended in a slightly basic solution (i.e., sterile TE buffer at pH 8). Exception: Cy® dye-labelled oligonucleotides should be resuspended at pH 7.
- We recommend preparing a stock solution at 100 µM concentration which can be achieved by adding a volume (µl) of sterile TE buffer or dH_2O, equal to ten times the number of nanomoles of sample present in the tube.
- (si)RNA should be resuspended in RNase-free buffer to a convenient stock concentration (20 to 50 µM) and in small aliquots to avoid multiple freeze thaw cycles and contact with RNases.

Storage and stability

- The below table describes oligonucleotide stability and storage recommendations, in a nuclease-free environment. For long-term high quality storage, prepare workable aliquots to avoid multiple freeze-thaw cycles (not more than 5).

Products	Format	Storage*	Stability**
Oligonucleotides	Dried	RT	18 months
	TE Buffer (pH 8) or dH_2O	-20°C	24 months
Real-Time qPCR Probes	Dried	RT	18 months
	TE Buffer (pH 8) or dH_2O	-20°C	24 months
RNAi Oligonucleotides	Dried	RT	18 months
	RNase-free Buffer (pH 7.5)	-20°C	24 months
Universal Primers	Dried	RT	18 months
PNA FISH Probes/ Custom PNA	Dried	RT	18 months

* Oligonucleotides and especially dye-labelled oligonucleotides should be protected from light for optimal stability. Tolerance -20°C ± 5°C.

** Please note that depending on sequence and modifications, the stability of the oligos may vary substantially versus the given values, which should therefore be considered as indicative. Although it is generally recommended to avoid freeze-thaw cycles, we did not observe negative effects in qPCR tests after 10 freeze-thaw cycles.

Oligonucleotide quantification

1 OD_{260} (Optical Density) unit is defined as the amount of oligonucleotide which, when dissolved in a volume of 1.0 ml, results in an absorbance of 1.0 when measured at 260 nm in a 1 cm path-length quartz cuvette. 1 OD_{260} unit corresponds to approximately 33 µg of single strand DNA. These relationships, however, can be inaccurate for short fragments of DNA, such as oligonucleotides. Base composition and even linear sequence will affect optical absorbance. Hence the precise value of the OD to mass relationship is unique for each oligo.

Example: 1.0 OD_{260} of CCCCCCCCCC (10 mers) equals 39 µg whereas 1.0 OD_{260} of AAAAAAAAAA (10 mers) equals only 20 µg

We carefully measure the OD value for your custom oligonucleotide by measuring the absorption at 260 nm using UV spectrophotometer. This information is provided on the oligonucleotide Technical Data Sheet as the number of OD_{260} units. The amount of oligo expressed in nanomoles and micrograms is derived from the OD measurement.

The following equation is used to calculate the number of nanomoles present given an OD reading and extinction coefficient:

$$\textbf{Nanomoles} = (OD_{260} / \varepsilon_{260}) \times 10^6$$

Example: 1 OD_{260} unit of primer M13 Forward, 5'-GTA AAA CGA CGG CCA GTG-3'
Molar extinction coefficient (ε_{260}) = 182,800 L / (mole x cm)
Nanomoles = $(1.0 / 182.800) \times 10^6$ = 5.47 nmolesThe following equation is used to convert from Nanomoles to Micrograms:

$$\textbf{Micrograms} = \textbf{Molecular Weight} \times \textbf{Nanomoles} \times 10^{-3}$$

Example: 1 OD_{260} unit of primer M13 Forward, 5'-GTA AAA CGA CGG CCA GTG-3'
Molecular Weight = 5558.7
Micrograms = $5558.7 \times 5.47 \times 10^{-3}$ = 30.4 µg

To quantify your oligonucleotide, make an aliquot of the resuspended oligonucleotide to a final volume of 1 ml of dH_2O and vortex for a few seconds. Measure the absorbance of this dilution at 260 nm (A_{260}). Use the formula below to calculate the concentration of oligonucleotide in your stock solution. This formula is valid for an absorption of $A_{260} \leq 1.2$.

Concentration in µg/ml =
A_{260} × dilution factor x Weight per OD of stock solution (in µg / OD)

Tm calculation

The melting temperature (Tm value) of an oligonucleotide is dependent upon the length of the sequence, the G+C content and the type and concentrations of cation present, particularly sodium ion, Na^+. We are using the following formulae to calculate the Tm:

- Recommended for primers from 14 to 20 bases :

$$Tm_1\ (°C) = 2 \times (A+T) + 4 \times (G+C) \quad \text{(Wallace-Ikatura formula)}$$

- Recommended for primers > 20 bases :

$$Tm_2\ (°C) = 81.5 + 16.6 \times \log_{10}[0.05] + 0.41 \times (\%G + \%C) - (675/N)$$
$$= (81.5 - 21.597098) + 0.41 \times (\%G + \%C) - (675/N)$$

where N is the length of the oligo. The formula we use takes into account the salt concentration of the reaction, as PCR is typically performed in the presence of ± 50 mM monovalent cations (0.05 in the above formula).

For degenerated oligos, the lowest (% G + % C) value must be used. For oligos containing Inosine, length = (Length of the oligo) - (Number of Inosine bases). Tm calculation is inaccurate for LNA®, PNA and may be inaccurate for oligos containing certain modified bases.

Calculation of the molar extinction coefficient

$$\varepsilon_{260} = 2 \times \left(\sum_{1}^{N-1} \varepsilon_{\text{Nearest Neighbour}} \right) - \sum_{2}^{N-1} \varepsilon_{\text{individual}} + \sum_{1}^{N} \varepsilon_{\text{Modification}}$$

where $\varepsilon_{\text{Nearest Neighbour}}$ is the nearest neighbour constant for a pair of bases, $\varepsilon_{\text{Individual}}$ is the constant for an individual base, and N is the length of the oligonucleotide. Please note that $\varepsilon_{\text{Modification}}$ is not known for all modifications.

Calculation of the molecular weight :

$$\textbf{Anhydrous MW (g/mol)} = \Sigma_{\text{Individual Base}} MW + \Sigma_{\text{Individual Mods}} MW - 63.98 + 2.016$$

For DNA bases: MW dA = 313.21; MW dC = 289.18; MW dG = 329.21; MW dT = 304.20; MW dU = 290.17; MW dI = 314.19

For RNA bases: MW DNA counterpart + 16
When determining the weight of Uracil (rU) start with dU and not dT

For 2' O-Methyl bases: MW DNA counterpart + 30.03.
When determining the weight of mU start with dU and not dT

For phosphorothioated bases: MW DNA counterpart + 16.06

Mixed bases

Mixed bases (also known as degenerate or wobble bases) follows the IUB codes: D=A/G/T, M=A/C, H=A/C/T, W=A/T, R=A/G, Y=C/T, V=A/C/G, S=C/G, K=G/T, N=A/G/C/T, B=C/G/T. Oligonucleotides made using mixed bases result in a final product that is a heterogeneous population of distinct species. MW, Tm and extinction coefficient may be strongly affected by mixed base addition. Rather than reporting the various values for each components, a single value is given.

These products are provided for research use only. They may not be used for commercial, clinical, diagnostic or any other use.

Alexa® is a registred trademark of Molecular Probes, Inc. • Alexa Fluor® is a registred trademark of Molecular Probes, Inc. • BHQ-0™, BHQ-1™, BHQ-2™ and BHQ-3™ are registred trademarks of Biosearch Technologies, Inc. • Black Hole Quencher™ is a registred trademark of Biosearch Technologies, Inc. • BODIPY® is a registred trademark of Molecular Probes, Inc. • CAL Fluor™ is a trademark of Biosearch Technologies, Inc. • Cascade blue® is a registred trademark of Molecular Probes, Inc. • Cy® is a registred trademark of GE Healthcare Bio-Sciences Corp. • Cy5® Direct is a registred trademark of GE Healthcare Bio-Sciences Corp. • LNA® is a registred trademark of Exiqon A/S. • Marina Blue® is a registred trademark of Molecular Probes, Inc. • OliGold® is a registred trademark of Eurogentec S.A. • Oregon Green® is a registred trademark of Molecular Probes Probes, Inc. • Pacific Blue™ is a trademark of Molecular Probes, Inc. • Plexor™ is a trademark of promega Corporation • Rhodamine Green™ is a trademark of Molecular Probes, Inc. • Rhodamine Red™ is a trademark of Molecular probes, Inc. • Texas Red® is a registred trademark of Molecular Probes, Inc. • Yakima Yellow® is a registred trademark of Epoch Biosciences, Inc.

GOL-DATASHEET-EU-0917-V7

Eurogentec
Experience true partnership