

Custom Oligo Specifications

Gene Link custom oligonucleotides are supplied desalted and lyophilized. They are ready to use after appropriate reconstitution. Dry oligonucleotides are stable at room temperature for an extended period of time.

Storage & Reconstitution

The oligonucleotide should preferably be frozen upon receipt. TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) is recommended for dissolving the oligonucleotides. After reconstitution store the stock solution at -80°C or -20°C.

Gel Photo Documentation

An actual gel picture of the synthesized custom oligonucleotide is supplied. A major single band represents high purity of the crude oligonucleotide.

Purity & Usage

The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50mer.

Biophysical Data

Each oligo after desalting is quantified by recording A_{260} . Exact nmols and μg is determined by the extinction coefficient and molecular weight of the oligo.

Oligo Scale of Synthesis and Typical Yield of Unmodified Oligos*

Scale	Crude Desalted		RPC Purified***		Gel Purified	
	20mer oligo**		30mer oligo**		50mer oligo**	
	A ₂₆₀ Units	nmols	A ₂₆₀ Units	nmols	A ₂₆₀ Units	nmols
50 nmol	8-10	30+	4-5	12+	NR* [1-2]	NR* [2-4]
200 nmol	20-25	80+	8-12	24+	4-6	8+
1 μmol	100-120	400+	40-50	30+	20-25	40+
Purity & Yield	Purity is more than 80% depending on oligo sequence and structure.		Purity 85% to 95% depending on oligo sequence and structure. Not recommended for oligos longer than 35mer.		Purity 98% to ~100% depending on oligo sequence and structure. Yield will gradually decrease as length of oligo increases.	

*The yield of modified oligos varies based on modification.

**Yield of 30 $\mu\text{g}/A_{260}$ unit for oligos is calculated for an ~equimolar base composition. Long stretches of a single base or homopolymers will have variable yields. Example for homopolymeric 50mer: A(50) ~20/ A_{260} Unit; G(50) ~28/ A_{260} Unit; T(50) ~35/ A_{260} Unit and C(50) ~39/ A_{260} Unit.

***RPC is reverse phase purification using a cartridge; a substitute for HPLC.

NR* Not Recommended.

Oligo Labeling

Oligo labeling for Non Radioactive Hybridization Probes & Affinity Chromatography

Radioactive labeling is still used extensively despite the development of sensitive non-radioactive detection methods. Chemiluminescent, visible dye and fluorescence-based detection offer a safe and sensitive method for qualitative, as well as quantitative detection.

Gene Link offers synthesis of non-radioactive modified oligos for use as hybridization probes and affinity chromatography. We provide technical service in the design of novel probes and synthesize numerous combinations of fluorescent dyes and quenchers, ligands, modifications and direct conjugation to alkaline phosphatase.

Sensitivity

Chemiluminescent detection with alkaline phosphatase approaches 0.1pg, equivalent to $\sim 1 \times 10^6$ copies of the target; this is equivalent to less than 4 μg of human genomic DNA. Almost all Southern based hybridizations can be switched to safe non-radioactive based methods with no associated hazard or need to frequently label probes.

Detection Strategy

The non-radioactive modification used must be complemented with a sensitive detection method. There are two approaches. In the direct method, the oligo probe is directly labeled with fluorescent dye(s) for fluores-

cent in situ hybridization (FISH); or with alkaline phosphatase (AP). The blot is incubated with an AP substrate that either gives visible color or chemiluminescence detected by exposure to X-ray film or imaging systems. In the indirect method, the probe is labeled with a ligand such as biotin or digoxigenin*. The blot is then incubated with an AP or horseradish peroxidase (HRP) conjugated antibody specific for the ligand followed by color or chemiluminescent detection.

Modifications

Oligos can be labeled with biotin or digoxigenin* or directly labeled with alkaline phosphatase. A variety of linker arms are available as spacers to minimize steric hindrance. See table for a list of popular ligand modifications and spacer molecules.

Signal Optimization

Placing three units of the label per oligo gives maximal sensitivity. One can be placed at each end and one in the middle with a minimum of 15 bases distance to prevent steric hindrance.

Fluorescence In Situ Hybridization (FISH)

Fluorescence In Situ Hybridization (FISH) is an important tool for the cell biologist and cytogeneticist. Using different combinations of dye-labeled oligos followed by in situ hybridization, provides a way to reliably detect target DNA, DNA insertions, deletions and translocations at the chromosomal level.

Affinity Chromatography

Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the

Oligo Modifications For Probes and Affinity Chromatography

Product	Description
Biotin TEG	Biotin with 16 atom spacer
5' or 3' biotinylation	Biotin with short 6 C spacer
Biotin dT	Biotinylated dT for internal labeling
5' or 3' amino linker (C3, C6 or C12)	Amino functional group with varying spacer arm for conjugation to ligands and enzymes
Digoxigenin	Hapten
Alkaline Phosphatase	Enzyme conjugation directly to amino modified oligos
Amino dT	Amino functional group on dT for conjugation to ligands and enzymes

This list does not include fluorescent dyes and other modifications. Please visit www.genelink.com for complete list.

purification of DNA binding proteins or cognate DNA molecules by specific hybridization based affinity chromatography. The biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography.

*Roche holds exclusive rights to digoxigenin labeling. Digoxigenin oligo labeling is offered under license from Roche. Extensive digoxigenin labeling techniques and detection methods are available from Roche.

Detection Methods

