## 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; EDTA inhibits the activity of the nucleases. Further dilution can be made in distilled sterile water. 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. Polyacrylamide gels of 12 to 15% are run, depending upon the length of the custom oligonucleotide. 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**

| | Crude Desalted | | | RPC Purified** | | | Gel Purified | | |
|---|---|---|---|---|---|---|---|---|---|
| | 20mer oligo* Typical yield | | | 30mer oligo* Typical yield | | | 50mer 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+ | 0.26-0.40 | 4-6 | 8+ | 0.13-0.2 |
| **1 µmol** | 100-120 | 400+ | 3-4 | 40-50 | 30+ | 1.3-1.6 | 20-25 | 40+ | 0.6-0.8 |
| **Purity & Yield** | Purity is more than 80% depending on oligo sequence and structure. Refer to coupling efficiency table for oligo length dependent purity and yield.<br>No further purification required for PCR and sequencing applications.<br>Gel purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis. | | | Purity 85% to 95% depending on oligo sequence and structure.<br>Yield and purity will be lower for sequences with high GC content.<br>Not recommended for oligos longer than 35mer.<br>**RPC is reverse phase purification using a cartridge; a substitute for HPLC. | | | Purity 98% to ~100% depending on oligo sequence and structure.<br>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 induces strong secondary structure and base stacking thus decreasing purity and yield.<br>NR* Not Recommended | | |

*Yield of 30µg/$A_{260}$ 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.

## Oligo Reconstitution and Use

Gene Link oligos are supplied lyophilized. These are stable at room temperature for an extended period of time. TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) is recommended for dissolving the oligonucleotides; EDTA inhibits the activity of the nucleases. Further dilution can be made in distilled sterile water. After reconstitution store the stock solution at –80°C or –20°C.

**Standard PCR Set Up**

| Reagent | Final Concentration | Quantity/ 50µl Reaction |
|---|---|---|
| Sterile deionized water | – | variable |
| 10X *PCR buffer | 1X | 5µl |
| 2mM dNTP mix | 0.2mM of each | 5µl |
| Primer I, 10µM (10pmol/µl) | 0.5µM | 2.5µl |
| Primer II, 10µM (10pmol/µl) | 0.5µM | 2.5µl |
| *Taq* DNA Polymerase, 5U/µl | 1.25u/50µl | 0.25µl |
| Template DNA | 10pg-1µg | variable |
| *Final $MgCl_2$ concentration is 1.5mM | | |

### Oligo Reconstitution

**Stock solution of 500pmols/µl [500µM]**

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 2 to arrive at the volume of TE to be added.

**Example:** 45.10nmols x 2 = 90.2µl

Dissolve the oligo in 90.2µl to get 500pmols/µl stock solution. Use as required.

Dilute 10 fold to prepare a 50pmols/µl [50µM]. Use as required.

**Stock solution of 100 pmols/µl [100µM]**

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of TE to be added.

**Example:** 45.10nmols x 10 = 451µl

Dissolve the oligo in 451µl to get 100pmols/µl stock solution. Use as required.

Dilute 10 fold to prepare a 10pmols/µl [10µM]. Use as required.

### Examples of Use

**Polymerase Chain Reaction (PCR)**

The final concentration of primers in a PCR reaction is 0.2–1.0µM. This is equivalent to 0.2–1pmol/µl. At Gene Link, for a standard PCR we use 0.5pmol/µl.

**Sequencing**

The final concentration of primer in automated sequencing is from 4 to 10pmols (~0.05 - 0.1µg). Use the oligo reconstitution protocol to prepare a 100pmols/µl [100µM] solution and then dilute 10 fold to get 10pmol/µl solution. Use 1µl (10pmols).

### Quick Conversion Table

1µM (µMolar) = 1pmol/µl (picomoles/µl)
1mM (milliMolar) = 1nmols/µl (nanomoles/µl)

**Example:** 20µMolar primer solution is 20pmol/µl

 

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