



Annealing of DNA oligonucleotides/Duplex Formation

While some manufacturers state that re-annealing of complementary DNA oligonucleotides is generally not necessary, we recommend to go through the process of duplex formation after having dissolved the delivered ds oligonucleotide prior to your experiments applying a suitable protocol.

If you ordered two single strand (ss) DNA oligos, separately dissolve both strands at a convenient stock concentration, e.g. at 100 μM (recommended) in DNase free water (pH 7<9). We recommend to prepare aliquots and store those, which are not used immediately at -20°C .

- Separately dilute one aliquot per DNA oligonucleotide to a final concentration of 50 μM using DNase free water.
- Mix 40 μl of each DNA oligonucleotide solution and add 20 μl of 5 x Annealing Buffer*, arriving at a final volume of 100 μL , and a final DNA duplex concentration of 20 μM .
- Incubate the solution for 1 min at $90-95^{\circ}\text{C}$ and gradually/slowly cool down to room temperature (over a period of no less than 30 min). Store at 4°C or on ice until ready to use. We recommend to process and use this re-annealed working solution within 24hours.

If you ordered DNA duplex, dissolve the delivered pellet in 1 x Annealing Buffer* at a convenient stock concentration, e.g. 100 μM . We recommend to prepare aliquots and store those, which are not used immediately, at -20°C .

- For duplex formation, dilute one ssDNA aliquot each to the concentration needed for your experiment (e.g. 20 μM) using 1 x Annealing buffer*.
- Incubate the solution for 1 min at $90-95^{\circ}\text{C}$ and gradually/slowly cool down to room temperature (over a period of no less than 30 min). Store at 4°C or on ice until ready to use. We recommend to process and use this re-annealed working solution within 24 hours.

Annealed DNA duplexes can be safely stored at -20°C . Do not freeze-thaw more than 5 times. We recommend to perform the re-annealing procedure described above before using thawed duplex aliquots.

*Annealing buffer:

5 x Annealing buffer concentration: 300 mM KCl, 30 mM HEPES-pH 7.5, 1.0 mM MgCl₂

1 x Annealing buffer concentration: 60 mM KCl, 6 mM HEPES-pH 7.5, 0.2 mM MgCl₂