Annealing oligonucleotides

protocol

For resuspending and annealing dried-down, complementary oligonucleotides to form a duplex

1. Centrifuge tubes before opening to ensure duplexed oligos are at the bottom of the tube.
2. Resuspend both oligos in Nuclease-Free Duplex Buffer* (Cat # 11-01-03-01) to reach the appropriate final volume. For example:

	100 µM final duplex concentration		50 µM final duplex concentration		10 µM final duplex concentration	
Oligo amount†	Buffer volume for oligos	Total volume for annealing	Buffer volume for oligos	Total volume for annealing	Buffer volume for oligos	Total volume for annealing
10 nmol	50 µL each	100 µL	100 µL each	200 µL	500 µL each	1 mL
25 nmol	125 µL each	250 µL	250 µL each	500 µL	1.5 mL each	2.5 mL
50 nmol	250 µL each	500 µL	500 µL each	1 mL	2.5 mL each	5 mL

† Refer to the IDT resuspension calculator at www.idtdna.com to calculate dilutions for other nanomole amounts.

3. Mix the two oligos in equimolar concentrations.
4. Heat at 94°C for 2 min.
5. Remove from heat and allow to cool to room temperature.
6. If needed, dilute the annealed oligonucleotides using Nuclease-Free Duplex Buffer or 1X IDTE Buffer (Cat # 11-01-02-05).
7. Store at –20°C.

Note: If the annealed oligos are to be used on multiple occasions, divide into smaller aliquots and store at –20°C.

* Nuclease-Free Duplex Buffer is certified nuclease-free by testing with RNaseAlert® and DNaseAlert™ reagents from IDT. For more information visit www.idtdna.com.