

Protocol for siRNA annealing

General Handling instructions:

The RNA–Oligonucleotides delivered by Bioneer are deprotected and purified. Although our Oligonucleotides are RNase free, RNA is highly susceptible to degradation by exogenous RNases introduced during handling. Therefore it is essential that all handling steps are conducted under sterile, RNase free conditions. RNA oligonucleotides should not be handled with ungloved hands. RNase free reagents, barrier pipette tips and tubes should be used.

Dry RNA oligonucleotides can be safely stored at -20°C for up to 6 months.

Annealing of siRNA:

Dissolve RNA oligonucleotides at a convenient concentration, e.g. $100\ \mu\text{M}$, in RNase free water. This solution should be stored at -20°C .

- Dilute each RNA oligonucleotide using annealing buffer to a final concentration of $50\ \mu\text{M}$.
- Combine $30\ \mu\text{l}$ of each RNA oligonucleotide solution and $15\ \mu\text{l}$ of annealing buffer. Final volume is $75\ \mu\text{l}$, final concentration of siRNA duplex is $20\ \mu\text{M}$.
- Incubate the solution for 1 minute at 90°C and cool slowly down afterwards to room temperature (over a period of ca. 45 min). Store at 4°C or on ice until ready to use.
- Annealed siRNA can be safely stored frozen at -20°C . Do not freeze–thaw more than 5 times.

Annealing buffer ingredients:

Annealing buffer concentration is: 30 mM HEPES–KOH pH 7.4, 100 mM KCl, 2 mM MgCl_2 , 50 mM NH_4Ac .