

Protocol for siRNA annealing and storage v2.0

A siRNA duplex is formed by hybridizing sense and antisense complementary RNA oligonucleotides.

RNA interference (RNAi) is a very promising new technology for gene silencing. Combining ease of use and efficiency, RNAi opens new horizons to study gene function, target validation and signal transduction.

RNAi is based on the introduction (by transfection or microinjection) of small double-stranded RNA called siRNA (for small interfering RNA). siRNA are typically made of 2 complementary synthetic strands containing 19 RNA bases followed by 2 DNA bases (preferably T). The preparation of a siRNA duplex is fast and simple as described in the following chapters.

General Handling Instructions

All reagents used in the synthesis and purification of Eurogentec's siRNA are certified DNase-RNase free. However, RNA oligonucleotides are highly susceptible to degradation by exogenous RNases introduced during handling. Therefore, it is crucial that all handling steps be conducted under sterile, RNase-free conditions. Please refer to suitable references for further instructions^{1,2}.

Upon receipt, dried RNA oligonucleotides may be safely stored in a non-frost free freezer for up to 6 months at -20 °C.

Resuspension of single-stranded siRNA oligonucleotides

Briefly centrifuge the tubes to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend oligonucleotides at a convenient concentration, e.g. 100 µM, in DEPC-treated water. This solution should be stored at -20 °C.

Annealing of siRNA

Scientists are sometimes puzzled when the time to choose the right protocol has come. Based on this observation, Eurogentec's RNA support team has summarized the best siRNA protocols in a simple and comprehensive document.

- Separately aliquot and dilute each RNA oligo to a concentration of 50 µM.
- Combine 30 µl of each RNA oligo solution and 15 µl of 5X annealing buffer.

Final buffer concentration is: 50 mM Tris, pH 7.5 - 8.0, 100 mM NaCl in DEPC-treated water. Note that some authors use a different annealing buffer (100 mM KAc, 30 mM HEPES-KOH pH 7.4, 2 mM MgAC final concentration)^{3,4}. No major difference has been observed when using this buffer.

Final volume is 75 µl. The final concentration of siRNA duplex is 20 µM.

- Incubate the solution for 1-2 minute in a water bath at 90-95 °C, and allow to cool to room temperature (i.e. below 30 °C) on your workbench. Centrifuge the tube briefly to collect all liquid at the bottom of the tube. Slow cooling to room temperature should take 45-60 minutes. Store on ice or at 4 °C until ready to use.
- Once annealed, duplex siRNA is much more nuclease resistant than single-stranded RNA and can be safely stored frozen at -20 °C in a non-frost free freezer. The annealing solution can be freeze-thawed up to 5 times.



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An alternative procedure for annealing involves the use of a thermal cycler.

Dispense appropriate volumes of each complementary RNA oligonucleotides, water and 5X buffer (see above) into PCR tubes (500 µl size). Do not overlay the samples with oil. Place the tubes in a thermal cycler and set up a program to perform the following program:

- (i) heat to 95 °C and remain at 95 °C for 2 minutes
- (ii) ramp cool to 25 °C over a period of 45 minutes
- (iii) proceed to a storage temperature of 4 °C.

Briefly centrifuge the tube in a microfuge to draw all droplets from the lid. Store on ice or at 4 °C until ready to use. The annealing solution can be stored frozen at -20 °C and freeze-thawed up to 5 times.

References

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2. Ausubel FM *et al.* Current Protocols in Molecular Biology. NY, Greene Pub. Associates and Wiley-Interscience (1987).
3. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*, 2001, 15(2):188-200.
4. Tuschl T, Zamore PD, Lehmann R, Bartel DP, Sharp PA. Targeted mRNA degradation by double-stranded RNA in vitro. *Genes Dev*, 1999, 13(24):3191-7.

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