



## Protocol for siRNA annealing

### General Handling instructions:

The RNA-Oligonucleotides delivered by metabion international AG 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  $-20^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$ .

- 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  $90^{\circ}\text{C}$  and cool slowly down afterwards to room temperature (over a period of ca. 45 min). Store at  $4^{\circ}\text{C}$  or on ice until ready to use.
- Annealed siRNA can be safely stored frozen at  $-20^{\circ}\text{C}$ . 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  $\text{MgCl}_2$ , 50 mM  $\text{NH}_4\text{Ac}$ .

### References:

[1] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, 2001, 411[6836]:494-8.

[2] Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*, 2001, 15[2]:188-200

[3] 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.

**Products for research only**

**Not for human use**

\*The inhibition of the expression of a given target gene by dsRNA may be protected by patent rights of Ribopharma AG. The use of certain RNAi fragments may be protected by patent rights of the Whitehead Institute for Biomedical Research, The Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V., the Massachusetts Institute of Technology, the University of Massachusetts Medical Center or other pending patents. To obtain a licence thereunder for your specific application of gene suppression with small RNA molecules, please contact the patent owners.