

# Protocol for RNA annealing/duplex formation

## General Handling instructions

RNA-oligonucleotides produced and delivered by metabion are **deprotected and HPLC purified by default**. Although our oligonucleotides are RNase free, RNA is highly susceptible to degradation by exogenous and ubiquitous RNases introduced during handling. Therefore, it is essential that all handling steps are performed under RNase free conditions. RNA oligonucleotides should not be handled without gloves. RNase free reagents, barrier pipette tips and tubes should be used.

Dry RNA oligonucleotides can be safely stored at  $-20\text{ }^{\circ}\text{C}$  for up to 18 months.

## Annealing of RNA/Duplex Formation

While some manufacturers state that re-annealing of RNA is generally not necessary we recommend to anneal RNA duplexes prior to your experiments using a suitable protocol (see below).

If you ordered two single strand (ss) RNAs, separately dissolve both RNA oligonucleotides at a convenient stock concentration, e.g. at  $100\text{ }\mu\text{M}$  (recommended), in RNase free water ( $\text{pH} >4 <9$ ). We recommend to prepare aliquots and store those, which are not used immediately at  $-20\text{ }^{\circ}\text{C}$ .

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

If you ordered an RNA duplex, dissolve the delivered pellet in 1 x **Annealing Buffer\*** at a convenient stock concentration, e.g.  $100\mu\text{M}$ . We recommend to prepare aliquots and store those, which are not used immediately, at  $-20\text{ }^{\circ}\text{C}$ .

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

Annealed RNA duplexes can be safely stored at  $-20\text{ }^{\circ}\text{C}$ . 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, suitable for siHE siRNA and others:

5 x Annealing Buffer concentration:  $100\text{ mM KCl}$ ,  $30\text{ mM HEPES-pH }7.3$ ,  $1\text{ mM MgCl}_2$

**(1ml of 5 x Annealing Buffer is provided free of charge with any siHE siRNA and RNA duplex order for your convenience)**

1 x Annealing Buffer concentration:  $20\text{ mM KCl}$ ,  $6\text{ mM HEPES-pH }7.3$ ,  $0.2\text{ mM MgCl}_2$

## Products for research use only

## 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 May 24;411(6836):494-8.
2. Elbashir SM., Lendeckel W., Tuschl T. **RNA interference is mediated by 21- and 22-nucleotide RNAs.** *Genes Dev*. 2001 Jan 15;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 Dec 15;13(24):3191-7.

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 license thereunder for your specific application of gene suppression with small RNA molecules, please contact the patent owners.