Product Protocol

SmartBase™ siRNA Modifications
Guaranteed RNAi Explorer kit with Molecular Probe
RNAi Explorer™ Online siRNA Design Algorithm
Custom siRNA Synthesis

SmartBase™ siRNA/RNA
Resuspension, Annealing, Handling and Storage

Modifications for Increased Cell Permeability, Duplex Stability & Nuclease Resistance

Duplex Stability & Nuclease Resistance Conferring Modifications

Propyne dC and dU
Phosphorothioate linkages
5-Me-dC & 2-amino dA
2'F bases
2'O methyl bases
2'MOE Bases
2'-5' linked Oligos
Methylated Oligos

Cell Delivery/Cell Permeation Modifications

Thiol & Amine
Cholesterol TEG
Spacer 18
Polyethylene Glycol (PEG)
Stearyl & α-tocopherol
GalNAc (N-acetylgalactosamine-C3)
Cell Penetrating Peptides (CPPs)

Gene Link™

RNA & SmartBase™ siRNA
Resuspension, Annealing, Handling and Storage

Gene Link supplies all RNA and siRNA oligos in a dried state and either annealed as duplex or single stranded as requested. These are DNase-RNase free and all steps in synthesis and processing are DNase-RNase free.

The exact nmols supplied is indicated on the Certificate of Analysis and the label on the tube. These can be requested as reconstituted at the desired concentration and choice of buffers.

Email Gene Link at support@genelink.com for any special requirements.

General Handling of RNA & siRNA oligos

RNA & siRNA oligos especially single-stranded are susceptible to degradation by RNase introduced during handling. Wear gloves and use RNase-free pipette tips and RNase free conditions.

The dried RNA and siRNA oligos can be safely stored in a non-frost-free freezer for up to 6 months at -20 °C.

Resuspension of RNA & siRNA oligos

The dried oligo during transportation may have dislodged from the bottom of the tube and may reside in the cap and/or distributed on the sides of the tube.

Prior to opening the tube, briefly centrifuge the tubes to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend single stranded siRNA oligonucleotides at a convenient concentration, e.g. 100 µM, in RNase free sterile water and duplex siRNA at a concentration of 100 µM. These are general guidelines, and you may elect to reconstitute at a different concentration or buffer condition.

Aliquot the siRNA into small volumes and store at –20°C to –80°C

Annealing of single-stranded siRNA

Reagents: Annealing buffer (5X)

Buffer 1:

50 mM Tris, pH 8.0
100 mM NaCl

Buffer 2:

100 mM Potassium Acetate
30 mM HEPES at pH 7.4
2 mM Magnesium Acetate

Note: Either Buffer can be used without much difference. All annealing solutions are given as 5X and can be stored frozen at -20ºC and freeze-thawed many times.

Annealing of single-stranded siRNA

1. Dissolve siRNA, as stated above, at a convenient concentration, e.g. 100 µM, in RNase free water and store at -20 °C or preferably at -80°C
2. Dilute each siRNA using sterile RNase free water to a final concentration of 50 µM.
3. Combine 30 µl of each siRNA solution and 15 µl of annealing buffer. Final volume is 75 µl, final concentration of siRNA duplex is 20 µM (30 µl X 50 µM = 75 µl X 20 µM).
4. Incubate the solution for 1 minute at 90 °C and cool slowly down afterwards to room temperature (over a period of about 45 min). This can be conveniently performed using a thermal cycler.
5. Briefly spin the tube to bring down all droplets from the wall and lid of the tube.
6. Aliquot the annealed siRNA into RNase-free tubes and store at -80°C. Do not freeze-thaw more than 5 times.

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.
4. http://www.rockefeller.edu/labheads/tuschl/sirna.html

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