Custom in vitro siRNA Design and Synthesis Services

Two pairs of target gene siRNA (~26µM x 100µl /pair)/\$559 Three pairs of target gene siRNA (~26µM x 100µl /pair)/\$699 Supplied with a negative control siRNA

Introduction:

Small interfering RNA (also named as short interfering RNA or silencing RNA) (siRNA) is a kind of double-stranded RNA molecules (20-25 nucleotides in length). They are identical to the sequence of target gene. Supplement of the double stranded RNA can suppress the target gene's expression through a process known as RNA interference (RNAi). This post-transcriptional process silences a gene through mRNA inhibition or degradation. siRNA technology is more and more popular method for gene specific inhibition in biomedical research.

Our siRNA synthesis service is based on *in vitro* transcription techniques and the silencer® siRNA Construction kit (Ambion). Dependent upon the custom gene sequence we will design two pairs of template oligonucleotides (sense and antisense), which contain 21 nt target gene encoding and a short sequence complementary to the T7 Promoter Primer. These two template oligonucleotides are then hybridized to a T7 Promoter Primer and extended by the Klenow fragment of DNA polymerase to create double-stranded siRNA transcription templates. These sense and antisense siRNA templates are subsequently transcribed by T7 RNA polymerase to create corresponding double-stranded RNA (dsRNA). After modification with single-strand specific ribonuclease and purification with glass fiber filter, the remained sequences with UU at 3'-terminal are the siRNAs. Those oligomers can be directly transfected into target cells to suppress corresponding gene expression.

Features and Benefits:

- Target gene suppression up to 95%. At lease 1 of 3 siRNA provides 80%-95% silencing in most mammalian cells.
- High quality 21-mer siRNA duplex is ready in fast turnaround times.
- Ready-to-use in vitro siRNA oligomers can be transfected directly into target cells.

[Necessary information and materials:]

Information from customer: Target protein (gene's name, gene's reference number, or mRNA sequence).

Transfection reagent: Lipofectamine 2000 (Invitrogen), Silencer® siRNA Transfection II Kit (Ambion), TransIT-TKO (Mirus), SuperFact (Qiagen), or other kind of transfection reagent (such as PEI, etc.)

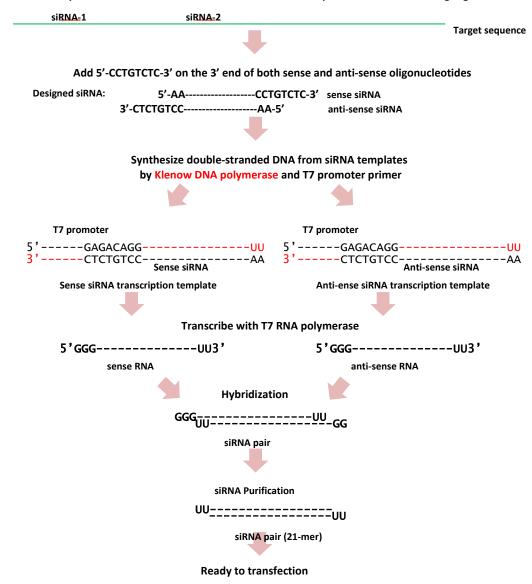
[Transfect procedure:]

The best working concentration of the siRNA for transfection is 0.1-10nM. The transfection processes can follow the product manual of the transfection reagent.

Please contact us at following e-mail: <u>Sam.lee@zmtechscience.com</u> for project quotation and timeline estimate.

Overview of siRNA design and synthesis procedure:

Computational search AA-head 21-nucleotide siRNA sequences based on the target gene



- 1. Two 29-mer DNA oligonucleotides are designed, synthesized and desalted.
- 2. A T7 Promoter Primer was added to the oligonucleotides and the 3'end extended by Klenow DNA Polymerase.
- 3. The sense and antisense siRNA template are transcribed by T7 RNA polymerase and hybridization.
- 4. RNase Digestion and DNA clean up.
- 5. The siRNA are further purified by glass fibre filters and the concentrations are optimized for transfection.
- 6. The high quality 21-mer siRNA duplex is ready to transfect into mammalian cells.

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