Instructions for Use of PromiseDown™ siRNA Oligo

Package Contents

PromiseDown™ siRNA Oligo provides expertly designed oligonucleotides for unmatched specificity and efficiency, ensuring precise and reliable gene silencing. With a broad selection of targets and flexible customization options, we help you push the boundaries of genetic research.

Click here to check your siRNA Oligo: https://tarmart.net/oligo

Product Type	Quality Control	Specification/Quantity
Gene-specific siRNA duplexes	Purified and sequence-verified	2.5nmol per vial 1 or 3 unique duplexes per gene
Negative control siRNA duplex	Purified and sequence-verified	2.5nmol per vial
Positive control siRNA duplex	Purified and sequence-verified	2.5nmol per vial
Fluorescent-labeled control siRNA duplex	Purified and sequence-verified	1nmol per vial

Recommended Transfection Reagents for siRNA Delivery

OligoMaxi™ RNA Transfection Reagent is a powerful siRNA/miRNA transfection reagent designed to ensure highly efficient and reproducible delivery of various small RNAs into mammalian cells.

OligoMaxi™ RNA Transfection Reagent Detail: https://tarmart.net/reagent/sirna-transfection-reagant

Storage Conditions

The dried duplexes can be stored at 4ºC. However, once reconstituted with DEPC-H2O, they must be stored at -20ºC. (2.5nmol siRNA add 25uL DEPC-H2O, 100nM)

Related products

#Lentivirus Plasmid

#plasmid

#Antibodies

Additional materials recommended

Transfection reagent: user-preferred (OligoMaxi recommended)
Cell line and cell culture supplies: user-preferred
Reagents for cell lysis: user-preferred
Reagents and supplies for immunoblotting: user-preferred

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited.
The siRNA kit contains a universal negative control RNA duplex (NC) that is absent in human, mouse, and rat genomes.

Product Application

Reconstitution of siRNA duplexes in duplex buffer or DEPC-H2O

1. Materials should only be handled with gloves under RNase-free conditions.
Briefly centrifuge each tube to ensure that all material is in the bottom of the tube and not in the cap before opening for the first time. Dried oligo often dislodges during shipping and can be lost.

2. Resuspend oligo duplexes: Resuspend duplexed oligos in Nuclease-Free Water to make a stock solution (concentration 100μM). For example:

Duplexed oligo amount	Nuclease-Free Water (100μM final concentration)
2.5nmol	25μL
10nmol	100μL
25nmol	250μL
50nmol	500μL

3. Make further dilutions (<100μM) using Nuclease-Free Water. For example:

Final concentration	100 μM duplexed oligo (from Step 2)	Nuclease-Free Water
50μM	25μL	25μL
20μM	20μL	80μL
10μM	10μL	90μL

Each 2.5nmol tube contains enough duplex for the number of transfections shown in Table

Plate Format	Media Volume (μL)/well	siRNA con. (10 nM)	siRNA con. (20 nM)
6 well	2000	1μL	2μL
12 well	1000	0.5μL	1μL
24 well	500	0.25μL	0.5μL
48 well	250	0.1μL	0.25μL
96 well	100	0.05μL	0.1μL

4. Once hydrated, duplexes should be stored at -20°C or -80°C. While generally stable to freeze/thaw cycles, GeneMedi recommends that daughter aliquots be made for routine use to minimize the frequency of freeze/thaw events for primary stock tubes. Minimize light exposure for dye-labeled duplexes.

Outline of an RNAi Experiment

1. Establish optimal transfection method for your cell type and culture media (use fluorescent-labeled transfection control duplex); favored approach is fluorescence microscopy. Greater than 90% of cells should show dye up take when examined 4-24 hours after transfection.

2. Demonstrate that RNAi is working using positive control (PC); favored approach is quantitative real-time RT-PCR.PC should show>90% knockdown 24 hours post-transfection at 20nM dose.

3. Test target specific duplexes and perform dose response curve. GeneMedi recommends testing duplexes at 20nM, 10nM, and 1nM concentrations. Knockdown of mRNA levels should be assayed at 24-48 hours post transfection. To limit off-target effects, routine studies should subsequently be performed using the lowest concentration of RNA duplex that achieves the desired level of suppression of the target mRNA.

4. Perform RNAi studies using duplexes identified as “effective by >70% reduction in RNA levels”. mRNA levels can generally be measured 24-48 hours post transfection. Protein levels can generally be measured at 48-72 hours post transfection, however this may vary depending on the half-life of the protein studied and cell growth rate.

5. Controls: While examination of non-transfection and mock-transfection cultures(lipid or electroporation alone) are useful, GeneMedi recommends that control cultures transfected using control RNA duplexes be used for target level normalization. A randomized sequence (Universal Negative Control) duplex is provided for this purpose, which is not present in human, mouse, or rat.

Transfection Optimization

Variables to consider for transfection optimization when using chemical reagents include:

1) Reagent choice (**OligoMaxi™ RNA Transfection Reagent**)
2) Cell density (>70%)
3) Ratio of reagent to siRNA (OligoMaxi : siRNA=1:1)
4) Amount of siRNA (siRNA final concentrations at 20nM, 10nM, and 1nM)
5) Length of time delivery reagent is left on cells
6) With some reagents, the transfection mixture can remain on the cells until harvest or passage with good results. Other reagents need to be washed from the cells or the cell death rate increases. A balance between maximal RNA knockdown and minimal cell death should be experimentally determined.
7) Presence or absence of serum in the medium
8) Post transfection incubation period

The length of incubation time post-transfection before cells are examined for RNAi effect varies with target and the assays employed. Fluorescence to assess transfection efficiency should be examined at around 24 hours post-transfection (within a 4–24 hour window).

RNA levels can usually be studied 24-48 hours after transfection. Protein levels and phenotypes are usually studied 48-72 hours after transfection; however the optimal time can vary depending on protein half-life, cell division rate, and a variety of other factors. Cells may need to be passaged to maintain healthy densities.

FAQs on the siRNA

Q1. What is the siRNA and how do they work?

siRNAs are short, noncoding, double-stranded RNAs that leverage the RNA interference (RNAi) pathway to achieve highly specific gene silencing. They suppress gene expression by targeting and selectively degrading complementary messenger RNA (mRNA), through RNA-induced silencing complex (RISC).

Q2. How do I order siRNAs?

You may order siRNAs through our website. The desired target can be searched by Symbol, Gene No, accession Number. Each query will yield three candidates. You may select the desired quantity at that point.

Q3. Can I order siRNAs targeted for a specific gene?

You may order siRNAs for the target gene of your choice by entering the gene name or Accession number at our website and selecting one of the listed siRNA candidates. You may also order custom designed siRNA if your model organism is not Human, Mouse or Rat. You can also order siRNAs by sequences, please email us.

Q4. What form will my order be in?

For siRNAs, both the sense and antisense strands are synthesized at equimolar concentrations, then annealed and delivered in duplexed, lyophilized form. EPDC-treated water with every order. We recommend reconstituting to 100μM.

Q5. I want to conduct an in vitro experiment. What scale should I choose? What purification should I select?

With a 2.5 nmol scale siRNA order, you can transfect ten 24-well plates at 20μM per transfection. For in vitro experiments, the standard small-scale purification method is OPC desalt. For in vivo experiments, HPLC purification is required. For in vivo experiments, HPLC purification is required.

Q6. I would like to know how the synthesized siRNA is purified.

To purify newly synthesized siRNA, we have several methods including OPC, HPLC and PAGE. We select a purification method depending on the end-use of the product:

In vitro experiments, OPC purification is enough for use.

in vivo experiments, HPLC is commonly used to reach the desired purity level. We use a reversed-phase resin for HPLC purification, and this will routinely yield up to 90% purity for 21-mer siRNA molecules.

Q7. Can GeneMedi synthesize chimeric RNA?

We have the ability to synthesize chimeric RNA molecules that incorporate DNA bases. We can also incorporate 2'-O-Methyl and 2'-F into the RNA.

Q8. What types of modified siRNAs are there?

The following modifications are offered for our siRNAs.

- 5' Amine - 3' amine modification
- 5' Phosphorylation & 3' phosphorylation
- 5' Thiol - 3' Thiol Modification
- 5' Biotin - Internal Biotin-dT Modification
- FAM modification

• BHQ modification
• Cy3 modification
• Cy5 modification
• Cy5.5 modification
• C16 modification ……

Q9. Are phosphate groups present on the 5' or 3' ends of the synthesized siRNA?

Unless explicitly stated, the 5' and 3' ends are capped with -OH groups. Therefore, to order 5' phosphate-capped siRNAs, you must request for 5' phosphorylation modification.

Q10. Up to how many bases can synthesize?

The maximum length for a single-stranded RNA (ssRNA) molecule is 45 bases. We charge per base for this type of order. For siRNA molecules, the maximum length is 30 bases, which is offered at a flat price.

Q11. What are precautions for handling siRNA?

- Wear gloves and a mask when handling siRNA.
- Always use RNase free tubes and pipette tips.
- Only use DEPC treated water when diluting siRNA.
- Store fluorescence-labeled siRNA in a dark location.

Q12. How should I store my siRNAs and how long do they keep?

siRNAs can normally be kept stable at -20°C for over 1 year. The lyophilized form is especially stable and has a long shelf-life. Although solution-dissolved siRNAs can be stable, contamination of the reconstitution solution with RNase will degrade the product. Also, repeated freeze-thaw cycles accelerate the degradation process. Therefore, we recommend that after you receive the siRNA stock, you reconstitute and make several aliquots to avoid such freeze-thawing.

Q13. How do I reconstitute my siRNA?

If you would like to keep your siRNA in solution, we recommend reconstituting with DEPC-treated water that we provide with your order to maximize stability.

Q14. How do I reach a target concentration?

Each synthesis report gives you a ' volume for 100pmol/μL' value that stands for the volume of DEPC-treated water you need to add to achieve a 100pmol/μL concentration. As an example, if the 'volume for 100pmol/μL ' values for two siRNAs are 100, then you can reconstitute with 100μl DEPC-treated water, respectively, to reach a 100pmol/μL concentration.

In other words, the siRNA contained in each tube is:

100μL x 100pmol/μL = 10,000pmol = 10 nmol,

Although the final use will dictate the concentration of siRNA, we found that for average use, 100pmol/μL is ideal. That is why we provide you with DEPC-treated water volume for reconstitution to 100pmol/μL.

Q15. How do I convert between molarity and moles?

The standard concentration units for oligomers is given in M (mol/L), and the prefixes such as μ (micro), n (nano), p (pico) etc. describe the scope of the unit. The following prefixes are used not only for M, but for other measurement units such as length, mass etc.

1pmol/μL = $1x10^{-12}$ mol / $1x10^{-6}$ L= $1x10^{-6}$ mol/L= 1μmol/L= 1μM

Therefore, μM and pmol/μL are one and the same.

Q16. Can I know how many ng the synthesized product is?

Normally, we will fulfill an order with a guaranteed nmol amount, and the synthesis report will also report the final amount in nmols. If you must have the ng amount to calculate for an experiment, you can convert from nmol to ng by using the formula below. We make it easy for you by giving you the molecular weight of the siRNA sequence in the report. Molecular Weight (g) X mole (nmol) = Mass of siRNA (ng)

Q17. This is my first siRNA experiment. How do I set my experimental conditions?

One of the most important factors in a siRNA experiment is the assessment of whether the siRNA gets delivered into the cell. GeneMedi offers positive controls that can easily indicate whether the siRNA is being delivered successfully.

Q18. To what cell density should I culture my cells before siRNA transfection?

For siRNA transfection, we recommend that the cell density be approximately 70%. (HEK293 cell, 6-well plate standard: 1.5 x 10^5 cells/well. We also strongly encourage user optimization of these figures)

Q19. What concentration of siRNA should I use for transfection?

We recommend a starting concentration of 20nM, but strongly advise empirical optimization for the cell line and conditions of your experiment.

Q20. How do I use 2.5nmol of siRNA to transfect cells at 20nM?

This is a source of confusion for many people. To transfect a single well with 20 nM siRNA, where the transfection volume is 2 mL, you will need 40 pmol of siRNA. Adding 2 μL of a 20 μM (20 pmol/μL) stock siRNA solution to 125 μL will yield 2.5 nmol of siRNA.

Q21. How should I store my fluorescent dye modified siRNA?

Photobleaching may occur if the fluorescent dye modified siRNA is exposed to light for prolonged periods of time. Therefore, we store such siRNAs in brown tubes and store that in a dark place. Dyes such as Cy3 and Cy5 are prone to degradation in solution environments where the pH is over 9. Therefore, avoid basic conditions when storing Cy3 and Cy5 modified siRNAs.

Q22. What transfection reagent do I use?

Because each cell line will have different transfection efficiencies for every transfection agent, we recommend that you select the transfection reagent most suitable for your cell line.

Q23. What controls are used for a siRNA experiment?

The Negative Control siRNA is a non-targeting siRNA that has low sequence homology to all known Human, Rat and Mouse sequences. Therefore, it can be used as a convenient negative control for all Human, Rat and Mouse siRNA experiments. The Positive Control siRNAs (human) demonstrate high knockdown efficiency for the target gene. Additionally, positive control siRNAs are available for reporter systems such as GFP and Luciferase.

Q24. How do I verify my siRNA transfection efficiency?

You can easily verify the transfection efficiency by transfecting your cells with NC-Cy3 and observing the cells with a fluorescence microscope. The NC-Cy3 can also be used as a test reagent to optimize the transfection concentrations of both the siRNA and the transfection reagent.

Q25. How do I verify the siRNA knockdown efficiency?

The siRNA knockdown efficiency can be verified through various techniques including qPCR, Western Blot.

Q26. The siRNA didn't work like I expected. What do I do?

Please verify the siRNA integrity with PAGE analysis. If the siRNA is intact, please verify the transfection efficiency. GeneMedi has a policy of supplying 3 free siRNAs if all 3 single-target-gene predesigned siRNAs have less than 70% knockdown efficiency, provided you supply us with the following information:

1. siRNA knockdown efficiency data 2. Transfection efficiency data

With the control:

NC: Negative Control,

PC: GAPDH/GFP/Luciferase Positive Control siRNA,

Cell transfection efficiency NC: Fluorescein labeled Negative Control

Q27. What are some precautions for a siRNA experiment?

First, because not all siRNAs will knock-down the target gene with identical efficiency, you should try 2-3 different sequences to find the best siRNA. Second, to make sure that the knock-down affects downstream protein expression, mRNA levels should also be measured. Thirdly, verify the knock-down phenotype by using another siRNA designed for the same target gene and show that the same phenotype appears.

Q28. When can I expect delivery?

Custom siRNA orders can be made and delivered in 10 business days.

Standard products are delivered in 5 business days.