PRODUCT INSERT

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Option A4 Custom siRNA Synthesis

Option A4 contents

RNA Oligos – The siRNA is shipped as a dried pellet in the 2'-deprotected and duplexed form. The oligos are ready for use immediately upon resuspension with RNase-free solutions. The mass of each oligo is confirmed by MALDI-TOF mass spectrometry.

Table 1. Recommended Resuspension Buffer Volumes and Final siRNA Concentrations

Synthesis Scale (µmol)	Amount per tube (nmol)	Total amount provided (nmol)	Amount of buffer to be added (mL)/tube	Final Concentration (µM= pmol/µL)
0.025	20	20	1.0	20
0.05	40	40	1.0	40
0.2	75	150	1.0	75
0.4	150	300	1.5	100
1.0	375	750	1.5	250

Option A4 protocol

1. Briefly centrifuge tubes containing siRNA to ensure that the siRNA pellet is collected at the bottom of the tube.
2. Resuspend siRNAs to a convenient stock concentration using the recommended volume of siRNA buffer shown in Table 1.
   - siRNA should be resuspended in RNase-free solutions. For example, an RNase-free buffer (pH 7.3-7.6) may be used such as PBS or 1x siRNA buffer (diluted from 5x siRNA buffer – Dharmacon Cat. #B-002000-UB-100). RNase-free water (for short-term storage) is also appropriate for resuspension of concentrated stocks (20-100 mM).
3. Pipette the solution up and down 3-5 times, avoiding the introduction of bubbles.
4. Place the solution on an orbital mixer/shaker for 30 minutes at room temperature.
   - This additional mixing results in more reliable resuspension as evidenced by OD260 readings.
5. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the solution is collected at the bottom of the tube.
6. Verify the concentration of siRNA using UV spectrophotometry (at 260 nM).
7. Aliquot the siRNA into small volumes and store at -20 °C to -80 °C. For best results, limit freeze-thaw events of each tube to no more than five.

Shipping and storage

- Oligo reagents are shipped as dry pellets at ambient temperature. Under these conditions, they are stable for atleast four weeks.
- Upon receipt, siRNA reagents should be stored at -20 °C to -80 °C. Under these conditions, the oligos are stable for at least one year.
- RNA should be resuspended in RNase-free solutions. For example, an RNase-free buffer (pH 7.3-7.6) may be used such as PBS or 1x siRNA buffer (diluted from 5x siRNA buffer – Dharmacon Cat. #B-002000-UB-100). RNase-free water (for short-term storage) is also appropriate for resuspension of concentrated stocks (20-100 µM).

Handling precautions

Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperature. We recommend wearing gloves and maintaining nuclease-free conditions when handling the oligonucleotides.

Supplemental documents

Go to dharmacon.horizondiscovery.com/resources/# to find:

- Product Information: MSDS, Protocols, and Product Literature
- Technical Resources: FAQs, Publications, and 2'- ACE chemistry Frequently Asked Questions

Frequently asked questions

Questions	Answers
How do I quantitate the resuspended siRNA?	RNA is most accurately quantified by measuring its absorbance at 260 nm (A_{260}) with a dual beam spectrophotometer.
How do I calculate the concentration of the siRNA sample?	Use Beer's Law, $A_{260} = (\varepsilon)(C)(L)$ where ε is the extinction coefficient (from the Product Transfer Form), C is the siRNA concentration, and L is the path length of the cuvette. Calculate the final concentration of the resuspended siRNA by solving for C and multiplying by the dilution factor.
Why does the calculated amount of RNA in solution differ from that on the Product Transfer Form?	Sample may not be homogeneously mixed. Upon drying, RNA may form aggregates or higher order structures. To disrupt, heat samples to 95 °C for 1-3 minutes and slow cool for 30-45 minutes to reanneal complementary strands. Differences in instrumentation for quantifying RNA may lead to differences in apparent values. Dual beam UV-VIS spectrophotometers are recommended.
The sample has been at room temperature for a week. Will the RNA still be okay?	Yes. Samples are shipped as dried pellets and are stable at room temperature for 2-4 weeks. Upon receipt, we recommend that all samples should be stored at -20 °C or -70 °C to -80 °C.
What is the average molecular weight of a siRNA?	The average molecular weight (MW) is 13,300 g/mol.
How do I convert between nmol to g of siRNA?	Use our nmol to µg calculator, or multiply the number of moles by the MW on the Product Transfer Form or the average MW, for 5 nmol of siRNA: $(5 \text{ nmol})(13{,}300 \text{ g/mol})(\text{mol}/10^9 \text{ nmol})(10^6 \text{ µg/g}) = 66.5 \text{ µg}$

For additional Frequently Asked Questions (FAQs), click here.

Supplemental products

RNase-free Water – RNase-free water is available for purchase at dharmacon.horizondiscovery.com, Cat #B-003000-WB-100, 100 mL.)

5x siRNA Buffer – 5x siRNA Buffer is compatible with most cell culture media. The final concentration of a 1x dilution is 60 mM KCl, 6 mM HEPES-KOH pH 7.5, 0.2 mM $MgCl_2$. (5x siRNA Buffer is available for purchase at dharmacon.horizondiscovery.com, Cat #B-002000-UB-100, 100 mL.)

If you have any questions, contact

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