PRODUCT INSERT

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INSPIRED CELL SOLUTIONS

Option A1 custom siRNA synthesis

Dharmacon option A1 contents

RNA oligos

The siRNA is shipped as single strands in 2'-ACE protected single-stranded form, these oligos will need to be 2'-ACE deprotected and desalted using the provided protocol, and purified by the end user. The mass of each oligonucleotide is confirmed by MALDI-TOF mass spectrometry.

2'-Deprotection buffer (1.8 mL)

The 100 mM Acetic Acid – TEMED pH 3.8 buffer effects removal of the Dharmacon 2'-ACE protecting groups and converts the RNA into the free 2'-OH form. The acid-catalyzed hydrolytic byproducts of the 2'-deprotection step are volatile and readily removed upon desalting.

3 M Sodium Acetate (1.5 mL)

The Sodium Acetate induces precipitation of the duplex.

Option A1 protocol

I. 2'-Deprotection and Annealing

1. Add 200 µL of 2'-Deprotection Buffer to each 2'-ACE protected, single-stranded complementary RNA strand. Combine the volumes of complementary strands of RNA, vortex and centrifuge.
2. Incubate the RNA at 60 °C in a dry heat block for 45 minutes.
3. Remove from heat and centrifuge briefly, 1–2 seconds.
4. Cool at room temperature for 30 minutes to form the RNA duplex.

II. Desalting the duplex

1. Add 50 µL of 3 M Sodium Acetate and 1.5 mL of 100% ethanol to the 400 µL of siRNA duplex solution.
2. Vortex 10 seconds.
3. Place the solution at –20 °C overnight or at –80 °C for 2 hours.
4. Centrifuge the solution at 13000 × g for 20 minutes at 4 °C.
5. Pour the supernatant from the tube.
6. Slowly pipette 200 µL of 95% Ethanol onto the pellet.
7. Pour the 95% Ethanol from the tube.
8. Dry the sample under vacuum with a lyophilizer or speed-vac.
9. The dry pellet can be stored at –20 °C until use or resuspended in an appropriately buffered RNase-free solution.

III. Quantitation of the siRNA duplex

To quantitate the RNA use Beer's Law: Absorbance (260 nM) = (ε)(concentration)(path length in cm), where ε is the molar extinction coefficient (provided on the Product Transfer Form supplied with the order).

When solved for the unknown, the equation becomes: Concentration = (Absorbance, 260 nM) / [(ε)(path length in cm)]. When a standard 10 mm cuvette is used, the path length variable in this equation is 1. If a different size of cuvette is used, such as a 2 mm microcuvette, then the path length variable is 0.2.

Table 1. Recommended resuspension buffer volumes and final siRNA concentrations.

Synthesis scale (µmol)	Number of tubes per strand	Amount per tube (nmol) (non-modified RNA)	Total amount provided (nmol)	Amount of buffer to be added (mL)/tube	Final Concentration (µM=pmol/µL)
.025	1	20	20	1.0	20
0.05	1	40	40	1.0	40
0.2	2	75	150	1.0	75
0.4	2	150	300	1.5	100
1.0	2	375	750	1.5	250

Shipping and storage

- Oligo reagents are shipped as dry pellets at ambient temperature. Under these conditions, they are stable for at least 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 https://dharmacon.horizondiscovery.com/resources to find:

- Product Information: SDS, Protocols, and Product Literature
- Technical Resources: FAQs, Publications, and 2'-ACE chemistry

Frequently asked questions

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.

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? Multiply the number of moles by the MW on the Product Transfer Form or the average MW, for 5 nmol of siRNA: (5 nmol)(13,300 g/mol)(mol/109 nmol)(106 µg/g) = 66.5 µg. Alternatively, use our easy online calculator.

For additional frequently asked questions (FAQs), click here.

Supplemental products

- RNase-free Water – RNase-free water is available for purchase, 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 MgCl2. (5x siRNA Buffer is available for purchase at. Cat #B-002000-UB-100, 100 mL.)

If you have any questions, contact

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