PRODUCT INSERT

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INSPIRED CELL SOLUTIONS

Option *in vivo* and *in vivo* HPLC Custom siRNA Synthesis

Dharmacon option *in vivo*

The siRNA is shipped as a lyophilized pellet in the 2'- deprotected and duplexed form. This processing option also includes counter ion (Na+) exchange, desalt, sterile filtration and endotoxin testing. The mass of each oligonucleotide is confirmed by ESI mass spectrometry and the siRNA is ready for use immediately upon resuspension.

Table 1a. (for unmodified siRNA) Recommended Resuspension Buffer Volumes and Final siRNA Concentrations:

Synthesis scale (µmol)	Amount per tube (nmol)	Total amount provided (nmol)	Amount of buffer per tube (mL)	Final concentration (µM= pmol/µL)
0.2	50	100	1.0	50
0.4	100	200	1.0	100
1.0	250	500	1.0	250

Dharmacon option *in vivo* HPLC

The siRNA is shipped as a lyophilized pellet in the 2'-deprotected and duplexed form. This processing option also includes reverse-phase HPLC purification of each oligonucleotide, counter ion (Na+) exchange, desalt, sterile filtration and endotoxin testing. The mass of each oligo is confirmed by ESI mass spectrometry and the minimum purity is 85% full length material. The siRNA is ready for use immediately upon resuspension.

Table 1b. (for unmodified siRNA) recommended resuspension buffer volumes and final siRNA concentrations:

Synthesis Scale (µmol)	Amount per tube (nmol)	Total amount provided (nmol)	Amount of buffer per tube (mL)	Final Concentration (µM= pmol/µL)
0.2	25	50	1.0	25
0.4	50	100	1.0	50
1.0	125	250	1.0	125

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.

Resuspension 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 buffer shown in (Table 1a) or (Table 1b) or based on the specific protocol to be used.
   • 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 (Fisher Cat. #BP399-500) RNase-free water (for short-term storage) is also appropriate for resuspension of concentrated stocks.
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 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

- siRNA reagents are shipped as dry pellets at room temperature (23 °C). 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 siRNA are stable for at least one year.
- siRNA should be resuspended in RNase-free solutions. RNase-free water (for short-term storage) is appropriate for resuspension of concentrated stocks (for example, 20-100 µM). Alternatively, an RNase-free buffer (pH 7.3-7.6) may be used such as PBS (Fisher Cat. #BP399-500).

Special note and disclaimer

This document provides general guidelines for the use of siRNA in whole animals. The end-user is ultimately responsible for the *in vivo* project design, and we make no guarantees regarding the use of these products or guidelines in whole animal studies.

Supplemental web resources

dharmacon.horizondiscovery.com/resources/#, Product Information: MSDS, Protocols, and Product Literature.
dharmacon.horizondiscovery.com/resources/#, Technical Resources: FAQs, Publications, and 2'ACE chemistry.

Frequently asked questions

Questions	Answers
What do I need to consider before initiating an *in vivo* study?	Experimental design for in vivo studies includes the choice of animal model, route of administration, dosage, and frequency of the dosing regimen. Recommendations for amounts or concentrations will depend largely on the nature of the target (tumor type, organ), its expression, and the size of the study.
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.
Is there a list of literature citations for *in vivo* applications?	A list of *in vivo* Dharmacon product literature citations is available in PDF format here. We encourage investigators to consult PubMed or HighWire for similar studies to determine specific delivery needs.

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

Related products

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

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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