GE Healthcare

Protocol

Dharmacon™ Basic siRNA Resuspension

This protocol is for the resuspension of synthetic siRNA

Note: This protocol is written for siRNA, but may also be applied to applied to microRNA mimic resuspension. For detailed recommendations for resuspension of siRNA/microRNA in plates, please see our siRNA Library Guidelines.

1. Briefly centrifuge tubes containing siRNA to ensure that the siRNA pellet is collected at the bottom of the tube.
2. Resuspend in RNase-free 1x siRNA Buffer (See note below) for the desired final concentration using volumes listed in Table 1.
   a. For example: for 10 nmol of siRNA and a 20 µM stock concentration, add 500 µL 1x siRNA Buffer.
3. Pipette the solution up and down 3-5 times, avoiding the introduction of bubbles and securely seal tubes (or multi-well plates).
4. Place the solution on an orbital mixer/shaker for 30 minutes at room temperature.
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. For siRNA, 1 µM = 13.3 ng/µL. For microRNA mimic, 1 µM=14.1 ng/µl, see FAQs for additional information.
7. RNA may be used immediately, or aliquoted into smaller volumes to limit the number of freeze-thaw cycles. Resuspended siRNA should be stored at -20 °C in a manual defrost or non-cycling freezer. Storage at 4 °C is suitable for up to 6 weeks.

Table 1. Recommended siRNA resuspension volumes and concentrations.

siRNA Amount (nmol)	1x siRNA Buffer to be added (µL) for desired final concentration	
	100 µM Stock	20 µM Stock
1.0	10	50
2.0	20	100
5.0	50	250
10	100	500
20	200	1000
50	500	Exceeds tube volume
100	1000	

Notes:

- **siRNA (and microRNA mimic) should be resuspended in RNase-free solutions. We recommend 1x siRNA Buffer (diluted from Dharmacon 5x siRNA Buffer, Cat #B-002000-UB-100). For short-term storage, RNase-free water (Cat # B-003000-WB-100) is also appropriate for resuspension of concentrated stocks.**
- **Salts present in buffer are known to affect the absorbance reading of RNA. For the most precise readings, dissolve in 4 volumes of sterile RNase-free water for spectrophotometric analysis. Then adjust with addition of 5x siRNA Buffer appropriately to desired final concentration of siRNA adjusting to 1x siRNA Buffer. Please see the FAQ section on page 2.**
- **To dilute the 5x siRNA Buffer to 1x siRNA Buffer, mix four volumes of sterile RNase-free water with one volume of 5x siRNA Buffer. The composition of the 1x siRNA Buffer is 60 mM KCl, 6 mM HEPES-pH 7.5, and 0.2 mM $MgCl_2$.**
- **5x siRNA Buffer is not intended for *in vivo* applications, as it has not been optimized for physiological conditions. Instead, an appropriately buffered RNase-free solution should be used.**

Technical Considerations:

- For efficient Dharmacon™ siGENOME™ siRNA, ON-TARGET*plus*™ siRNA, or miRIDIAN™ microRNA Mimic delivery, we strongly recommend following the instructions provided by the manufacturer for the delivery method of choice (such as transfection reagent, or electroporation) and taking measures to test and optimize the conditions best suited for the cell line or culture selected. For protocols using DharmaFECT™ transfection reagents or Dharmacon™ Accell™ siRNA delivery, click here.
- Assays for mRNA level, protein level, or phenotypic change may be performed to assess silencing effects. Because RNAi is an mRNA-specific event, we highly recommend assaying for reduction at the mRNA level using reverse transcription quantitative real-time PCR (RT-qPCR). Typical time points for detecting target knockdown with lipid-mediated siRNA or microRNA mimic delivery are 24-48 hours for mRNA and 48-96 hours for protein. Accell siRNA delivery is typically assessed at 72 hours or longer. Time course studies are recommended to identify optimal time points for assessing knockdown.

Frequently Asked Questions:

Question	Answer
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?	Salts present in 1x siRNA Buffer (or other resuspension solution) are known to cause a decrease in the absorbance reading of RNA. Differences in instrumentation for quantifying RNA may lead to differences in apparent values. Dual beam UV-VIS spectrophotometers are recommended. Sample is too concentrated. Absorbance values are most accurate between 0.15 and 0.6 and within the linear range of a standard curve. Sample is too diluted. Measurements with dilutions of small volumes (1-1.5 µL) are more susceptible to variation. Sample may not be fully resuspended. Heat samples to 95 °C for 1-3 minutes and allow to cool for 30-45 minutes to reanneal complementary strands.
The siRNA has been at room temperature for a week. Will the siRNA 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 or miRIDIAN™ Mimic?	The average molecular weight (MW) of a siRNA is 13,300 g/mol. The average MW of a miRIDIAN mimic is 14,100 g/mol.
How do I convert between nmol to µg of siRNA?	Multiply the number of moles by the MW on the Product Transfer Form, or the average MW for your oligo. For example, 5 nmol of siRNA would be: $(5\ nmol)(13{,}300\ g/mol)(mol/10^9\ nmol)(10^6\ \mu g/g) = 66.5\ \mu g$

For additional Frequently Asked Questions (FAQs), please visit here.

Orders can be placed at:
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1.800.235.9880; 303.604.9499 if you have any questions.

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