Guidelines and Recommendations for Dharmacon™ siRNA Libraries

Handling and storage recommendations

Dharmacon siRNA reagents are shipped as dry pellets at ambient temperature and should be stored at -20 °C upon arrival in a manual defrost or non-cycling freezer. Under these conditions, the siRNAs are stable for at least one year. If necessary, siRNAs can be stored as as dry pellets (unopened) at 4 °C for several weeks.

Resuspension recommendations

1. Briefly centrifuge plates to ensure that the siRNA is collected at the bottom of the well.
2. Wipe adhesive foil cover with 70% ethanol or other RNase-decontamination solution such as Fisherbrand™ RNase Displace™ Decontaminant (Cat #04-355-136; 04-355-138; 04-355-137).
3. Carefully peel back the foil seal to gain access to wells. Use caution and avoid shredding the seal.
4. Dilute 5x siRNA buffer (Cat #B-002000-UB-100) to 1x concentration (resuspension buffer) with RNase-free water before resuspending siRNA. RNase-free water is available from the Dharmacon product catalog (Cat #B-003000-WB-100).
   Note: For optional siRNA quantification by UV spectrophotometry (at 260 nm), resuspend well(s) in four volumes of RNase-free water. Following this analysis, add 1 volume 5x buffer for appropriate final 1x concentration. Salts present in buffer are known to cause a decrease in the absorbance reading of RNA. For additional tips on accurate spectrophotometry readings, please see the FAQ section of our website.
5. Resuspend siRNAs to a convenient stock concentration using the recommended volume of 1x resuspension buffer or RNase-free water shown in Table 1. Concentrated stocks of 20 µM or more are recommended. However, stock solutions of 1-10 µM may better accommodate dilution schemes for high-throughput transfections and assays conducted on robotic platforms.
6. Pipette solution up and down 3-5 times while avoiding introduction of bubbles.
7. Place the solution on an orbital mixer/shaker for 70-90 minutes at room temperature. This additional mixing results in more reliable resuspension.
8. Briefly centrifuge plates to collect solution to bottom of the wells.
9. siRNA may now be used immediately, stored at -20 °C (4 °C is suitable for 4-6 weeks) in a manual defrost or non-cycling freezer, or aliquoted into daughter plates.

a. Polypropylene accommodates storage at -80 °C and is often used for daughter plate creation.
b. Polystyrene plates are suitable for -20 °C storage, but become brittle at -80 °C and may be subject to breakage.

10. Seal plates with appropriate adhesive or heat seal.
11. Limit freeze-thawing of each plate. Up to 15 freeze thaws can be tolerated but for best results, limit these events to no more than five. Under these conditions, the siRNA is stable for at least 6 months.

siRNA Amount (nmol)	1x resuspension buffer to be added (µL) for desired final concentration		
	2 µM Stock	10 µM Stock	20 µM Stock
0.1	50	n/a	n/a
0.25	125*	25	n/a
0.5	250*	50	25
1.0	500	100	50
2.0	1000	200	100

*this volume will exceed the capacity of a well in a 384-well plate (max 120 µL).
n/a: volumes required to resuspend at this concentration are too low for efficient reconstitution

Plate types and layout:

96-well siRNA libraries

- NUNC Polystyrene 96 well V- bottom plates (Cat #249952)
- Agilent Peelable Aluminum Seal (Cat #24210-001, white) **OR** Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738, silver)
- Catalog 96-well libraries are fulfilled with the following plate layout: 80-wells per plate, columns 1 and 12 left empty "1" refers to siRNA reagent to gene 1, "2" refers to siRNA reagent to gene 2, etc.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Empty	1	2	3	4	5	6	7	8	9	10	Empty
B	Empty	11	12	13	14	15	16	17	18	19	20	Empty
C	Empty	21	22	23	24	25	26	27	28	29	30	Empty
D	Empty	31	32	33	34	35	36	37	38	39	40	Empty
E	Empty	41	42	43	44	45	46	47	48	49	50	Empty
F	Empty	51	52	53	54	55	56	57	58	59	60	Empty
G	Empty	61	62	63	64	65	66	67	68	69	70	Empty
H	Empty	71	72	73	74	75	76	77	78	79	80	Empty

384-well siRNA libraries

- Thermo Scientific™ ABgene™ 384-Well Storage Plate (Polypropylene, Pyramidal bottom) (Cat #AB-0781)
- Agilent Peelable Aluminum Seal (Cat #24210-001, white) **OR** Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738, silver)
- Catalog 384-well libraries are fulfilled with one of the following plate layouts: a) 280-wells per plate, rows A and P, plus columns 1, 2 and 23, 24 left empty **OR**
  b) 320-wells per plate, columns 1, 2 and 23, 24 left empty
  NOTE: Please refer to the platemap provided with order for your precise layout, and contact Technical Support with any questions.

Cherry-pick libraries

Requests for customer-specified lists of pre-designed siRNA and/or microRNA reagents can be fulfilled directly on the Dharmacon website with the Cherry-pick Library Plater. The addition of controls and customization of plate layout is under control of the user.

Cherry-pick Libraries can be generated for siRNA reagents from a list of any of the following identifiers:

- Official Gene Symbol e.g. BRCA1, CDC2, YF13H12
- Gene ID e.g. 983 (CDC2)
- Dharmacon Catalog number e.g. L-040411-00

Information provided with all library orders

Plate Maps are provided in Excel files via USB drive and include Sample Location (Plate and Well), Catalog Number, Gene Symbol, Gene ID, RefSeq Accession Number, and Sequence Information.

Frequently asked questions (FAQs)

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, 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. The RNA may not be fully resuspended. Dilute further by adding more volume of water or buffer. Heat samples to 56 °C for 5 minutes, and allow to cool to room temperature.
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, miRIDIAN™ Mimic , or miRIDIAN Hairpin Inhibitor?	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. The average MW of a miRIDIAN hairpin inhibitor is 18,500 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: $(5\ nmol)(13{,}300\ g/mol)(mol/10^9\ nmol)(10^6\ \mu g/g) = 66.5\ \mu g$.

View additional Frequently Asked Questions (FAQs)

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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