

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 dry pellets (unopened) at 4 °C for several weeks.

Resuspension recommendations

- Briefly centrifuge plates to ensure that the siRNA is collected at the bottom of the well.
- 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).
- Carefully peel back the foil seal to gain access to wells. Use caution and avoid shredding the seal.
- 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.
- 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.

- Pipette solution up and down 3-5 times while avoiding introduction of bubbles.
- Place the solution on an orbital mixer/shaker for 70-90 minutes at room temperature. This additional mixing results in more reliable resuspension.
- Briefly centrifuge plates to collect solution to bottom of the wells.
- 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.
 - Polypropylene accommodates storage at -80 °C and is often used for daughter plate creation.
 - Polystyrene plates are suitable for -20 °C storage, but become brittle at -80 °C and may be subject to breakage.
- Seal plates with appropriate adhesive or heat seal.
- 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.

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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