

Guidelines and Recommendations for miRIDIAN™ microRNA Libraries

Handling and Storage Recommendations:

Dharmacon™ miRIDIAN™ 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 mimics and hairpin inhibitors are stable for at least one year. If necessary, dry RNA pellets (unopened) can be stored at 4 °C for several weeks.

Resuspension Recommendations:

1. Briefly centrifuge plates to ensure that the RNA pellet 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. Pierce or carefully peel back the foil seal to gain access to wells. Use caution and avoid shredding the seal.
4. Dilute 5x siRNA resuspension buffer (Cat #B-002000-UB-100) to 1x concentration with RNase-free water before resuspending miRIDIAN products. RNase-free water is available from Dharmacon (Cat #B-003000-WB-100).

Note: For optional RNA quantitation by UV spectrophotometry (at 260 nm), resuspend well(s) in 4 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 mimics or inhibitors to a convenient stock concentration using the recommended volume of 1x siRNA 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. Mimics or inhibitors 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 Thermo Scientific™ ALPS™ 3000 Microplate Heat Sealer (Cat #AB-3000) or other 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 mimics and inhibitors are stable for at least 6 months.

Table 1.

Amount per well (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	n/a	100	50
2.0	n/a	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 or exceed the capacity of a 96-well plate.



Plate Types and Layout:

96-well miRIDIAN microRNA libraries

- NUNC Polystyrene 96 well V- bottom plates (Cat #249952)
- Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738)
- Default plate layout: 80-wells per plate, columns 1 and 12 left empty

"1" refers to mimic or inhibitor for microRNA 1, "2" refers to mimic or inhibitor for microRNA 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 miRIDIAN microRNA libraries

- Thermo Scientific™ Abgene™ 384-Well Storage Plate (Polypropylene, Pyramidal bottom) (Cat #AB-0781)
- Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738)
- Default plate layout: 280-wells per plate, rows A and P, plus columns 1, 2 and 23, 24 left empty

"1" refers to mimic or inhibitor for microRNA 1, "2" refers to mimic or inhibitor for microRNA 2, etc.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	Empty	Empty	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Empty	Empty
C	Empty	Empty	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Empty	Empty
D	Empty	Empty	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	Empty	Empty
E	Empty	Empty	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	Empty	Empty
F	Empty	Empty	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	Empty	Empty
G	Empty	Empty	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	Empty	Empty
H	Empty	Empty	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	Empty	Empty
I	Empty	Empty	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	Empty	Empty
J	Empty	Empty	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	Empty	Empty
K	Empty	Empty	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	Empty	Empty
L	Empty	Empty	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	Empty	Empty
M	Empty	Empty	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	Empty	Empty
N	Empty	Empty	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	Empty	Empty
O	Empty	Empty	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	Empty	Empty
P	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Custom Plate Layout Requests:

Catalog Libraries can only be fulfilled with the above plate layouts, since they are created from a single set of master plates. Alternative plate layouts are available for customer-defined Dharmacon™ Cherry-pick™ libraries, however differential pricing will apply to fulfill these requests and turnaround time will increase.

Cherry-pick Libraries:

Requests for customer-specified lists of pre-designed 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 microRNA reagents from a list of any of the following identifiers:

- miRBase ID e.g. hsa-miR-181b-5p
- Mature accession e.g. MIMAT0000257
- Dharmacon Catalog number e.g. C-311794

For questions, please contact our Technical Support staff.

Information provided with all Library orders:

Plate Maps are provided in Excel files via CD and include Sample Location (Plate and Well), Catalog Number, Mature Sanger ID, Precursor Accession Number, Mature Accession Number, and Mature Sequence.

Whole Genome Library information:

Dharmacon miRIDIAN Mimic and Hairpin Inhibitor Libraries are available for human, mouse and rat microRNAs in the miRBase sequence database, version 21.0.

When unique precursors give rise to identical microRNA mature sequences, those redundant reagents are accounted for in the annotated plate map file that accompanies the library. In this manner, the library does not contain multiple wells with identical reagents.

Mimics	96-well Catalog #	# Reagents	# 96-well Plates	384-well Catalog #	# 384-well Plates
Human miRIDIAN miRNA Mimic Library	CS-001040	2603	33	CS-001040-E2	10
Mouse miRIDIAN miRNA Mimic Library	CS-002040	1939	26	CS-002040-E2	8
Rat miRIDIAN miRNA Mimic Library	CS-003040	766	11	N/A	
Inhibitors					
Human miRIDIAN miRNA Inhibitor Library	IH-001040	2609	33	IH-001040-E2	10
Mouse miRIDIAN miRNA Inhibitor Library	IH-002040	1938	26	IH-002040-E2	8
Rat miRIDIAN miRNA Inhibitor Library	IH-003040	768	11	N/A	

Troubleshooting:

For technical information or troubleshooting contact Dharmacon GE Healthcare Molecular Biology Technical Support.

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GE Healthcare

Orders can be placed at:
gelifesciences.com/dharmacon

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Technical Support: ts.dharmacon@ge.com or
1.800.235.9880; 303.604.9499 if you have any questions.