

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).
- Pierce or 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 Thermo Scientific™ ALPS 3000™ Microplate Heat Sealer (Cat #AB-3000) or other 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.

Table 1.

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)
- Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738)
- 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)
- Thermo Scientific™ Easy Pierce™ Heat Seal (Cat #AB-3738)
- Catalog 384-well libraries are fulfilled with the following plate layout:
280-wells per plate, rows A and P, plus columns 1, 2 and 23, 24 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	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

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.

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Orders can be placed at:
gelifesciences.com/dharmacon

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