

SWIFT AMPLICON 384-PLEX WORKFLOW

Swift Amplicon™ 96-Reaction Panels

AL-51696, Swift Amplicon 16S+ITS Panel (96 rxns)

AL-56296, Accel-Amplicon 56G Oncology Panel v2 (96 rxns)

AL-50096, Accel-Amplicon Sample_ID Panel (96 rxns)

Swift Amplicon™ 384-Plex Combinatorial Dual (CD) Indexing Kits

Protocol for Cat. Nos.:

AL-S1A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1A, 96 rxns)

AL-S1B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1B, 96 rxns)

AL-S2A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2A, 96 rxns)

AL-S2B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2B, 96 rxns)

Visit swiftbiosci.com/protocols for updates.

About This Guide

This guide provides information and instructions for use of the Swift Amplicon Combinatorial Dual (CD) Indexing Kits (384 reactions) with the 96-reaction kit size of the following panels:

- AL-51696, Swift Amplicon 16S+ITS Panel (96 rxns)
- AL-56296, Accel-Amplicon 56G Oncology Panel v2 (96 rxns)
- AL-50096, Accel-Amplicon Sample_ID Panel (96 rxns)

Please Note: These panels do not include indexing kits but are otherwise identical to the standard 48 reaction Accel-Amplicon and Swift Amplicon Panels.

The following Swift Amplicon Combinatorial Dual Indexing kits must be purchased separately:

- AL-S1A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1A, 96 rxns)
- AL-S1B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1B, 96 rxns)
- AL-S2A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2A, 96 rxns)
- AL-S2B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2B, 96 rxns)

The indexing kits are provided as 96 unique combinations to enable flexibility for 96, 192, 288 and 384-Plex sequencing depending on the number of kits utilized.

Swift Amplicon Combinatorial Dual Indexing Kits are also compatible with the following panels. However, please note that these panels support only 48 reactions each and also include the standard D501-508/D701-712 Combinatorial Dual indexed adapters for 8x12 (96) multiplexing:

- AL-51648, Swift Amplicon 16S+ITS Panel (48 rxns)
- AL-56248, Accel-Amplicon 56G Oncology Panel v2 (48 rxns)
- AL-50048, Accel-Amplicon Sample_ID Panel (48 rxns)
- AL-55048, Accel-Amplicon CFTR Panel (48 rxns)
- AL-52048, Accel-Amplicon BRCA1 and BRCA2 Panel (48 rxns)
- AL-57048, Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel (48 rxns)
- AL-53048, Accel-Amplicon Comprehensive TP53 Panel (48 rxns)
- AL-51048, Accel-Amplicon EGFR Pathway Panel (48 rxns)
- AP-PC8048, Accel-Amplicon Plus 57G Pan-Cancer Profiling Panel (48 rxns)
- AP-CR8048, Accel-Amplicon Plus Colorectal Cancer Panel (48 rxns)
- AP-LN8048, Accel-Amplicon Plus Lynch Syndrome Panel (48 rxns)
- AP-LG8048, Accel-Amplicon Plus Lung Cancer Panel (48 rxns)
- AP-MY8048, Accel-Amplicon Plus Myeloid Panel (48 rxns)
- Accel-Amplicon Custom Panels

Please inquire at orders@swiftbiosci.com for 384 indexing with any Accel-Amplicon panel.

IMPORTANT!

This protocol addendum is designed to be used in conjunction with the full protocol for the [Swift Amplicon 16S+ITS Panel](#) or [Accel-Amplicon Panels](#) (56G and Sample_ID Panel).

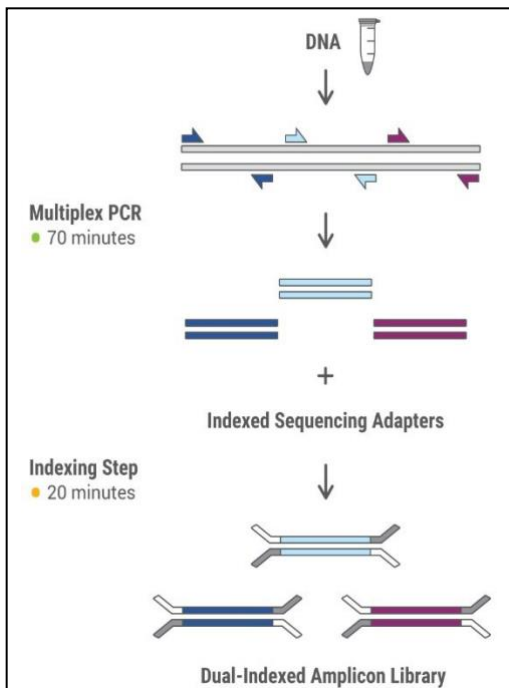
Applications and Product Information

Swift Amplicon Panels enable the preparation of high quality targeted next-generation sequencing (NGS) libraries from a variety of sample types. The single-tube workflow can be completed within two hours. The Swift Amplicon Combinatorial Dual Indexing Kits enable multiplexing of up to 384 libraries using a combinatorial dual indexing strategy (8 unique i5 indexes x 48 unique i7 indexes).

Swift Amplicon Panel Workflow

This protocol contains a Multiplex PCR step for the simultaneous production of amplicon targets in a single tube and an Indexing step for the addition of dual indexed adapters, enabling multiplexing of up to 384 unique libraries.

Bead-based clean-ups are used to purify the sample by removing unused oligonucleotides and changing buffer composition between steps.



Kit Contents

The Swift Amplicon Panels listed below are available in a package size with reagents (10% excess volume) for the preparation of 96 libraries. Reagents are provided in two tubes of 48 reactions each, as shown in the table below. Index kits are sold separately.

AL-51696, Swift Amplicon 16S+ITS Panel (96 rxns)

AL-56296, Accel-Amplicon 56G Oncology Panel v2 (96 rxns)

AL-50096, Accel-Amplicon Sample_ID Panel (96 rxns)

Kit Box	Reagents	Quantity (µl) 96 rxn	Storage (°C)
Multiplex PCR (Pre-PCR)	<input type="checkbox"/> Reagent G1*	2 x 106	-20
	<input type="checkbox"/> Reagent G2	2 x 160	-20
	<input type="checkbox"/> Enzyme G3	2 x 800	-20
	<input type="checkbox"/> Pre-PCR TE	2 x 1200	-20
Indexing Step (Post-PCR)	<input type="checkbox"/> Buffer Y1	2 x 1637	-20
	<input type="checkbox"/> Enzyme Y2	2 x 53	-20
	<input type="checkbox"/> Enzyme Y3	2 x 53	-20
	<input type="checkbox"/> Enzyme Y4	2 x 106	-20
	<input type="checkbox"/> Post-PCR TE	2 x 1200	-20

*Reagent G1 is the panel-specific set of multiplex amplification primers.

Additional Components Included

Reagent	Quantity (µl) 96 rxns	Storage (°C)
PEG NaCl Solution	20,000	Room Temp

Each of the four combinatorial dual index kits contain adapters with sequences listed in the **Appendix**. Index kits are available in a package size with reagents (10% excess volume) for the preparation of 96 libraries. Each kit includes D501-508 and a unique set of S7 adapters:

AL-S1A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1A, 96 rxns), Indexes S701-S712

AL-S1B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 1B, 96 rxns), Indexes S713-S724

AL-S2A96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2A, 96 rxns), Indexes S725-S736

AL-S2B96, Swift Amplicon Combinatorial Dual Indexing Kit (Set 2B, 96 rxns), Indexes S737-S748

Reagents	Quantity (μ l) 96 rxn	Storage ($^{\circ}$ C)
<input type="checkbox"/> Index D50X	66 each of D501-D508	-20
<input type="checkbox"/> Index S7XX	88 each of S701-S712 (Set 1A)	-20
<input type="checkbox"/> Index S7XX	88 each of S713-S724 (Set 1B)	-20
<input type="checkbox"/> Index S7XX	88 each of S725-S736 (Set 2A)	-20
<input type="checkbox"/> Index S7XX	88 each of S737-S748 (Set 2B)	-20

CHANGES TO THE PROTOCOL

IMPORTANT!

This protocol addendum is designed to be used in conjunction with the full protocol for the [Swift Amplicon 16S+ITS Panel](#) or [Accel-Amplicon Panels](#) (56G and Sample_ID Panel).

Multiplex PCR Step

There are no changes to this portion of the protocol. Please refer to the [Swift Amplicon 16S+ITS Panel](#) or [Accel-Amplicon Panels](#) (56G and Sample_ID Panel) protocol for panel specific thermocycling.

During thermocycling, it is recommended to plan and pre-aliquot your indexing combinations for step 16 below (See Appendix for plate layout)

Size Selection and Clean-Up Step 1

There are no changes to this portion of the protocol.

Indexing Step

Replace the protocol steps as follows for best results. In addition, it is recommended to plan and pre-aliquot your indexing combinations prior to the size selection and clean-up step (See Appendix for plate layout):

14. Load the Indexing Thermocycler Program and allow the block to reach 37 °C before loading samples.
15. Add 35 µl of the cold Indexing Reaction Mix to each sample and resuspend the pellet.
16. Add a unique combination of 5 µl Index D50X + 10 µl Index S7XX to each sample and mix thoroughly.

Addition of reagents in this order is recommended to avoid over-drying of beads.

17. Place in the thermocycler and run the program (37 °C for 20 minutes).

Size Selection and Clean-Up Step 2

There are no changes to this portion of the protocol

APPENDIX

Indexed Adapter Sequences

During the Indexing Step in the protocol, you must use a combination of Index Adapters to label each library. Libraries made with combinatorial dual indexed adapters may be multiplexed during cluster generation and co-sequenced on the same Illumina® flow cell. The number on the product tube label indicates which indexed adapter is provided in the tube. During library preparation, make sure to note which indexed adapter combination you are using with your sample and do not use the same indexed adapter combination on two different samples you plan to co-sequence.

CONTENTS: Combinatorial indexed adapters. There are eight different i5 indexes and 48 different i7 adapters, which can be used together to achieve 384 individually dual indexed library combinations.

i5 Adapters	Sequence for MiSeq, HiSeq® 2000/2500	Sequence for MiniSeq, NextSeq, HiSeq 3000/4000
D501	TATAGCCT	AGGCTATA
D502	ATAGAGGC	GCCTCTAT
D503	CCTATCCT	AGGATAGG
D504	GGCTCTGA	TCAGAGCC
D505	AGGCGAAG	CTCGCCT
D506	TAATCTTA	TAAGATTA
D507	CAGGACGT	ACGTCTTG
D508	GTA CTGAC	GTCAGTAC

NOTE: Use the reverse complement sequences provided in the table above when using Illumina MiniSeq, NextSeq, or HiSeq 3000/4000 systems.

i7 Adapters	Sequence	Set
S701	CAACACAG	Set 1A AL-S1A96
S702	ACACCTCA	
S703	ACCATAGG	
S704	CAGGTAAG	
S705	AACGCACA	
S706	TAGTCTCG	
S707	CAGTCACA	
S708	CCAACACT	
S709	ACATGCCA	

S710	ATTCCGCT	
S711	CAAGGTAC	
S712	CCATGAAC	
S713	TCAGCCTT	
S714	CAGTGCTT	
S715	CTCGAACA	
S716	ACAGTTCG	
S717	ATCCTTCC	
S718	CGAAGTCA	Set 1B
S719	CTCTATCG	AL-S1B96
S720	ACTCTCCA	
S721	TCCTCATG	
S722	AACAACCG	
S723	CTCGTTCT	
S724	TCAGTAGG	
S725	GCTTCACA	
S726	CGATGTTT	
S727	TTAGGCAT	
S728	ACAGTGGT	
S729	GCCAATGT	
S730	CAGATCTG	Set 2A
S731	ACTTGATG	AL-S2A96
S732	TAGCTTGT	
S733	TGGTTGTT	
S734	TGTACCTT	
S735	TCTGCTGT	
S736	TTGGAGGT	
S737	TCGAGCGT	
S738	TGATACGT	
S739	TGCATAGT	
S740	TGCGATCT	
S741	TTCCTGCT	
S742	TACAGGAT	Set 2B
S743	TGTGGTTG	AL-S2B96
S744	TTCCATTG	
S745	TAACGCTG	
S746	TTGGTATG	
S747	TGAACTGG	

If you experience problems with your library prep, please contact us at TechSupport@swiftbiosci.com, or by phone at 734.330.2568 (9:00 am-5:00 pm ET, Monday-Friday).

AL-S1A96, Swift Amplicon CD Indexing Kit (Set 1A, 96 rxns)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	501/704	501/705	501/706	501/707	501/708	501/709	501/710	501/711	501/712
B	502/701	502/702	502/703	502/704	502/705	502/706	502/707	502/708	502/709	502/710	502/711	502/712
C	503/701	503/702	503/703	503/704	503/705	503/706	503/707	503/708	503/709	503/710	503/711	503/712
D	504/701	504/702	504/703	504/704	504/705	504/706	504/707	504/708	504/709	504/710	504/711	504/712
E	505/701	505/702	505/703	505/704	505/705	505/706	505/707	505/708	505/709	505/710	505/711	505/712
F	506/701	506/702	506/703	506/704	506/705	506/706	506/707	506/708	506/709	506/710	506/711	506/712
G	507/701	507/702	507/703	507/704	507/705	507/706	507/707	507/708	507/709	507/710	507/711	507/712
H	508/701	508/702	508/703	508/704	508/705	508/706	508/707	508/708	508/709	508/710	508/711	508/712

AL-S1B96, Swift Amplicon CD Indexing Kit (Set 1B, 96 rxns)												
	13	14	15	16	17	18	19	20	21	22	23	24
A	501/713	501/714	501/715	501/716	501/717	501/718	501/719	501/720	501/721	501/722	501/723	501/724
B	502/713	502/714	502/715	502/716	502/717	502/718	502/719	502/720	502/721	502/722	502/723	502/724
C	503/713	503/714	503/715	503/716	503/717	503/718	503/719	503/720	503/721	503/722	503/723	503/724
D	504/713	504/714	504/715	504/716	504/717	504/718	504/719	504/720	504/721	504/722	504/723	504/724
E	505/713	505/714	505/715	505/716	505/717	505/718	505/719	505/720	505/721	505/722	505/723	505/724
F	506/713	506/714	506/715	506/716	506/717	506/718	506/719	506/720	506/721	506/722	506/723	506/724
G	507/713	507/714	507/715	507/716	507/717	507/718	507/719	507/720	507/721	507/722	507/723	507/724
H	508/713	508/714	508/715	508/716	508/717	508/718	508/719	508/720	508/721	508/722	508/723	508/724

AL-S2A96, Swift Amplicon CD Indexing Kit (Set 2A, 96 rxns)												
	25	26	27	28	29	30	31	32	33	34	35	36
A	501/725	501/726	501/727	501/728	501/729	501/730	501/731	501/732	501/733	501/734	501/735	501/736
B	502/725	502/726	502/727	502/728	502/729	502/730	502/731	502/732	502/733	502/734	502/735	502/736
C	503/725	503/726	503/727	503/728	503/729	503/730	503/731	503/732	503/733	503/734	503/735	503/736
D	504/725	504/726	504/727	504/728	504/729	504/730	504/731	504/732	504/733	504/734	504/735	504/736
E	505/725	505/726	505/727	505/728	505/729	505/730	505/731	505/732	505/733	505/734	505/735	505/736
F	506/725	506/726	506/727	506/728	506/729	506/730	506/731	506/732	506/733	506/734	506/735	506/736
G	507/725	507/726	507/727	507/728	507/729	507/730	507/731	507/732	507/733	507/734	507/735	507/736
H	508/725	508/726	508/727	508/728	508/729	508/730	508/731	508/732	508/733	508/734	508/735	508/736

AL-S2B96, Swift Amplicon CD Indexing Kit (Set 2B, 96 rxns)												
	37	38	39	40	41	42	43	44	45	46	47	48
A	501/737	501/738	501/739	501/740	501/741	501/742	501/743	501/744	501/745	501/746	501/747	501/748
B	502/737	502/738	502/739	502/740	502/741	502/742	502/743	502/744	502/745	502/746	502/747	502/748
C	503/737	503/738	503/739	503/740	503/741	503/742	503/743	503/744	503/745	503/746	503/747	503/748
D	504/737	504/738	504/739	504/740	504/741	504/742	504/743	504/744	504/745	504/746	504/747	504/748
E	505/737	505/738	505/739	505/740	505/741	505/742	505/743	505/744	505/745	505/746	505/747	505/748
F	506/737	506/738	506/739	506/740	506/741	506/742	506/743	506/744	506/745	506/746	506/747	506/748
G	507/737	507/738	507/739	507/740	507/741	507/742	507/743	507/744	507/745	507/746	507/747	507/748
H	508/737	508/738	508/739	508/740	508/741	508/742	508/743	508/744	508/745	508/746	508/747	508/748

Please use the plate layout above to generate 384 unique index combinations

Material Safety Data Sheet

Please refer to the product [material safety data sheet \(MSDS\)](#) for more information about the potential hazards for each component and instructions on safe use.

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Notes



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