

# Quick-16S™ Plus NGS Library Prep Kit (V4)



Catalog Nos. D6430-PS1, D6430-PS2, D6430-PS3, D6430-PS4  
**Guide for Preparing More than 96 Samples**

## Notice

One Quick-16S™ Plus NGS Library Prep Kit (V4) (96 rxns) can support the preparation of up to 96 samples for sequencing on the Illumina MiSeq® platform. The kit comes with Premix Plate containing Primer Set 1, which consists of unique dual index primers. To prepare more than 96 samples (up to 384 samples), multiple kits with different unique dual index primer sets must be ordered as described in the section below.

## Ordering Guidelines

The table below provides ordering information for customers who would like to prepare more than 96 samples with the Quick-16S™ Plus NGS Library Prep Kit (V4).

Anticipated Number of DNA Samples to Prepare	Recommended Cat #s to Order	Included Primer Sets
1-96	D6430-PS1	Primer Set 1
97-192	D6430-PS1 & D6430-PS2	Primer Sets 1 & 2
193-288	D6430-PS1, D6430-PS2, & D6430-PS3	Primer Sets 1, 2, & 3
289-384	D6430-PS1, D6430-PS2, D6430-PS3, & D6430-PS4	Primer Sets 1, 2, 3, & 4

## Protocol

For each group of up to 96 samples, use a single Quick-16S™ Plus NGS Library Prep Kit (V4) with the appropriate group of Index Primers as described in the protocol below. Samples 1-96 are assigned to Plate 1, samples 97-192 to Plate 2, samples 193-288 to Plate 3, and samples 289-384 to Plate 4.

1. Follow the protocol in **Section 1: 1-Step PCR** as written in the Quick-16S™ Plus NGS Library Prep Kit (V4) Instruction Manual (found on the D6430 Product Page at [www.zymoresearch.com](http://www.zymoresearch.com)). Use the diagrams below to add the appropriate sample to each premixed reaction, based on the plate number/sample group:

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Plate 1, Samples 1-96  
V4 Primer Set 1: ZB0001-ZB0096

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	POS*
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	NEG**

\* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

\*\* NEG: A no template control as a negative control.

Plate 2, Samples 97-192  
V4 Primer Set 2: Index Primers ZB0097-ZB0192

	1	2	3	4	5	6	7	8	9	10	11	12
A	S97	S105	S113	S121	S129	S137	S145	S153	S161	S169	S177	S185
B	S98	S106	S114	S122	S130	S138	S146	S154	S162	S170	S178	S186
C	S99	S107	S115	S123	S131	S139	S147	S155	S163	S171	S179	S187
D	S100	S108	S116	S124	S132	S140	S148	S156	S164	S172	S180	S188
E	S101	S109	S117	S125	S133	S141	S149	S157	S165	S173	S181	S189
F	S102	S110	S118	S126	S134	S142	S150	S158	S166	S174	S182	S190
G	S103	S111	S119	S127	S135	S143	S151	S159	S167	S175	S183	POS*
H	S104	S112	S120	S128	S136	S144	S152	S160	S168	S176	S184	NEG**

\* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

\*\* NEG: A no template control as a negative control.

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## Plate 3, Samples 193-288

V4 Primer Set 3: Index Primers ZB0193-ZB0288

	1	2	3	4	5	6	7	8	9	10	11	12
A	S193	S201	S209	S217	S225	S233	S241	S249	S257	S265	S273	S281
B	S194	S202	S210	S218	S226	S234	S242	S250	S258	S266	S274	S282
C	S195	S203	S211	S219	S227	S235	S243	S251	S259	S267	S275	S283
D	S196	S204	S212	S220	S228	S236	S244	S252	S260	S268	S276	S284
E	S197	S205	S213	S221	S229	S237	S245	S253	S261	S269	S277	S285
F	S198	S206	S214	S222	S230	S238	S246	S254	S262	S270	S278	S286
G	S199	S207	S215	S223	S231	S239	S247	S255	S263	S271	S279	POS*
H	S200	S208	S216	S224	S232	S240	S248	S256	S264	S272	S280	NEG**

\* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

\*\* NEG: A no template control as a negative control.

## Plate 4, Samples 289-384

V4 Primer Set 4: Index Primers ZB0289-ZB0384

	1	2	3	4	5	6	7	8	9	10	11	12
A	S289	S297	S305	S313	S321	S329	S337	S345	S353	S361	S369	S377
B	S290	S298	S306	S314	S322	S330	S338	S346	S354	S362	S370	S378
C	S291	S299	S307	S315	S323	S331	S339	S347	S355	S363	S371	S379
D	S292	S300	S308	S316	S324	S332	S340	S348	S356	S364	S372	S380
E	S293	S301	S309	S317	S325	S333	S341	S349	S357	S365	S373	S381
F	S294	S302	S310	S318	S326	S334	S342	S350	S358	S366	S374	S382
G	S295	S303	S311	S319	S327	S335	S343	S351	S359	S367	S375	POS*
H	S296	S304	S312	S320	S328	S336	S344	S352	S360	S368	S376	NEG**

\* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

\*\* NEG: A no template control as a negative control.

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2. **Section 2: Pooling by Equal Volume.** Add 50 µl of **PCR Inactivation Solution** into a new microcentrifuge tube. Pool equal volumes (**2 µl**) of PCR products from each well of all plates from Section 1 into the tube and mix well.
3. Follow the protocol in **Section 3: Final Library Clean-up** as written in the Instruction Manual.
4. Quantify the cleaned library with a fluorometric quantification method that uses dsDNA binding dyes, such as Qubit® as written in **Section 4: Library Quantification**.
5. The ultra-pure pooled library DNA is now ready for use or storage at ≤-20°C. Refer to platform-specific guidelines for preparation for sequencing. Custom sequencing primers included need to be spiked into the Illumina® reagent cartridge.
  - a. The MiSeq® Reagent Kit v2 (300-cycle) with 15% PhiX spike-in is recommended. See Appendix D in the Instruction Manual for assistance with sample sheet setup. Remember to set the index size to 10 bp.