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# Dual Indexes for MicroPlex Kit v3

High Performance Library Preparation for Illumina® NGS Platforms

- Cat. No.** C05010003 (24 Dual Indexes - 48 rxns)  
C05010004 (96 Dual Indexes - Set I - 96 rxns)  
C05010005 (96 Dual Indexes - Set II - 96 rxns)  
C05010006 (96 Dual Indexes - Set III - 96 rxns)  
C05010007 (96 Dual Indexes - Set IV - 96 rxns)



Please read this manual carefully  
before starting your experiment

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# Introduction

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Dual indexes for MicroPlex kits are designed for use with MicroPlex Library Preparation Kit v3 to construct libraries for multiplexed sequencing on Illumina® sequencers. These kits contain indexed PCR primers carrying the Illumina Nextera® XT v2 index sequences and offer a total of 384 dual indexes for multiplexing of up to 384 samples. All dual indexes kits are presented in Table 1.

Table 1. Dual Indexes for MicroPlex v3

Product	Cat. No.	Format	Concentration	Volume per well or tube
24 Dual indexes for MicroPlex Kit v3	C05010003	Individual Tubes	5 µM	10 µl
96 Dual indexes for MicroPlex Kit v3 - Set I	C05010004	Plate	5 µM	5 µl
96 Dual indexes for MicroPlex Kit v3 - Set II	C05010005	Plate	5 µM	5 µl
96 Dual indexes for MicroPlex Kit v3 - Set III	C05010006	Plate	5 µM	5 µl
96 Dual indexes for MicroPlex Kit v3 - Set IV	C05010007	Plate	5 µM	5 µl

**SHIPPING AND STORAGE:** The above kits are shipped on dry ice. The kits should be stored at -20°C upon arrival.

The Dual Indexes for the MicroPlex Kit v3 are designed for use with the following kits:

- MicroPlex Library Preparation Kit v3 x48 rxns (Cat. No. C05010001)
- MicroPlex Library Preparation Kit v3 x96 rxns (Cat. No. C05010002)

Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the Dual indexes for MicroPlex v3 kits.

# Remarks before starting

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## General using information

No more than four freeze/thaw cycles are recommended for the Dual Indexes for MicroPlex kits v3.

Prior to use, remove the kit from the freezer and thaw for 10 min on the bench top. Spin in a tabletop centrifuge to collect contents at the bottom of the tubes or wells. Thoroughly wipe the tubes or index plate foil seal with 70% ethanol and allow it to dry.

Follow standard laboratory safety procedures and wear a suitable lab coat, protective goggles and disposable gloves to ensure personal safety as well as to limit potential cross contamination during the sample preparation and subsequent amplification reactions.

## Multiplexing and Index Pooling

It is very important to select appropriate dual indexes that are unique and meet the Illumina recommended compatibility requirements. For low-plex (2- to 12-plex) pooling guidelines, please refer to Illumina's Nextera Low Plex Pooling Guide (1000000041074 v06).

# Dual index sequences

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The dual indexes for MicroPlex v3 are 8-nt long and use the Illumina Nextera XT v2 i5 and i7 dual index sequences (Table 2). Each indexing reagent tube (24 dual indexes kit) or well (96 dual indexes kits) contains a unique combination of i7 and i5 index primers. The below tables presents the sequences of i5 and i7 index primers.

Table 2. Sequences of i7 index primers

i7 index	i7 Bases for Sample Sheet
N701	TAAGGCGA
N702	CGTACTAG
N703	AGGCAGAA
N704	TCCTGAGC
N705	GGACTCCT
N706	TAGGCATG
N707	CTCTCTAC
N710	CGAGGCTG
N711	AAGAGGCA
N712	GTAGAGGA
N714	GCTCATGA
N715	ATCTCAGG
N716	ACTCGCTA
N718	GGAGCTAC
N719	GCGTAGTA
N720	CGGAGCCT
N721	TACGCTGC
N722	ATGCCGAG
N723	TAGCGCTC
N724	ACTGAGCG
N726	CCTAAGAC
N727	CGATCAGT
N728	TGCAGCTA
N729	TCGACGTC

Table 3. Sequences of i5 index primers

i5 index	i5 Bases for Sample Sheet (MiSeq®, NovaSeq, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
S502	CTCTCTAT	ATAGAGAG
S503	TATCCTCT	AGAGGATA
S505	GTAAGGAG	CTCCTTAC
S506	ACTGCATA	TATGCAGT
S507	AAGGAGTA	TACTCCTT
S508	CTAAGCCT	AGGCTTAG
S510	CGTCTAAT	ATTAGACG
S511	TCTCTCCG	CGGAGAGA
S513	TCGACTAG	CTAGTCGA
S515	TTCTAGCT	AGCTAGAA
S516	CCTAGAGT	ACTCTAGG
S517	GCGTAAGA	TCTTACGC
S518	CTATTAAG	CTTAATAG
S520	AAGGCTAT	ATAGCCTT
S521	GAGCCTTA	TAAGGCTC
S522	TTATGCGA	TCGCATAA

## Multiplexing and index pooling

It is very important to select appropriate dual indexes that are unique and meet the Illumina recommended compatibility requirements. For low-plex (2- to 12-plex) pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (1000000041074 v06) as shown in the table below.

Table 4. Index pooling guidelines

Plexity	Index 1 (i7) Adapters	Index 2 (i5) Adapters
2 - 6	At least 2 unique i7 adapters	At least two unique i5 adapters
7 - 12	One of the following combinations: N701, N702, N704 and any other i7 adapter N703, N705, N706 and any other i7 adapter	N505 and N506
More than 12	N701–N706 and any other i7 adapter	N505, N506, and any other i5 adapter



# 24 Dual indexes for MicroPlex v3 (C05010003)

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Dual indexing reagents are pre-dispensed in 24 individual tubes, each containing a different dual index combination. Each tube contains sufficient volume for up to two uses.

Each of the tubes in the 24 Dual indexes for MicroPlex Kit v3 contains a unique combination of i7 and i5 index primers (Table 5) carrying the 8-nt dual index sequences shown in Table 2 and 3.

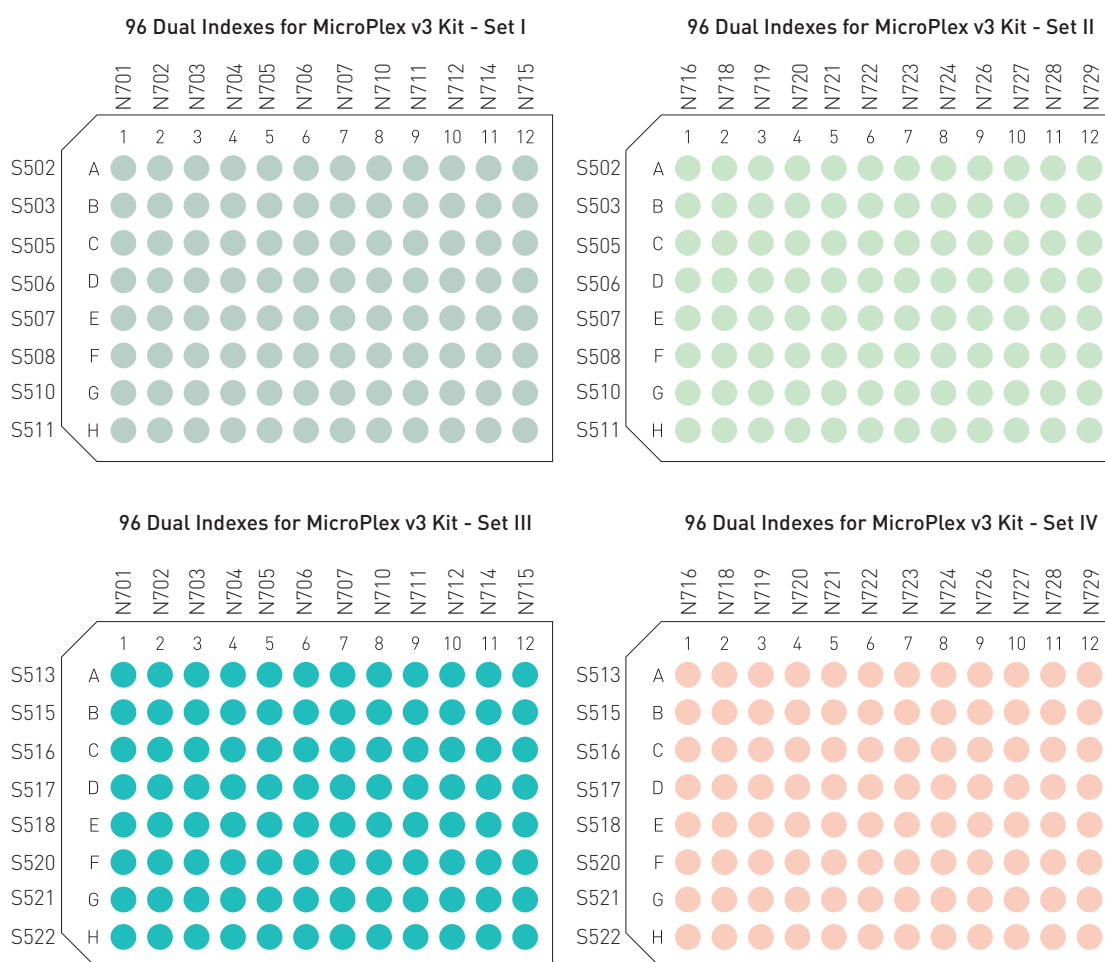
Table 5. Combination of i7 and i5 indexes in the 24 Dual indexes for MicroPlex Kit v3 (Cat. No. C05010003)

Dual Indexing Reagent	i7 index	i5 index
Dual Indexing Reagent 1	N701	S505
Dual Indexing Reagent 2	N702	S505
Dual Indexing Reagent 3	N703	S505
Dual Indexing Reagent 4	N704	S505
Dual Indexing Reagent 5	N705	S505
Dual Indexing Reagent 6	N706	S505
Dual Indexing Reagent 7	<b>N701</b>	<b>S506</b>
Dual Indexing Reagent 8	<b>N702</b>	<b>S506</b>
Dual Indexing Reagent 9	<b>N703</b>	<b>S506</b>
Dual Indexing Reagent 10	<b>N704</b>	<b>S506</b>
Dual Indexing Reagent 11	<b>N705</b>	<b>S506</b>
Dual Indexing Reagent 12	<b>N706</b>	<b>S506</b>
Dual Indexing Reagent 13	N701	S508
Dual Indexing Reagent 14	N702	S508
Dual Indexing Reagent 15	N703	S508
Dual Indexing Reagent 16	N704	S508
Dual Indexing Reagent 17	N705	S508
Dual Indexing Reagent 18	N706	S508
Dual Indexing Reagent 19	<b>N701</b>	<b>S517</b>
Dual Indexing Reagent 20	<b>N702</b>	<b>S517</b>
Dual Indexing Reagent 21	<b>N703</b>	<b>S517</b>
Dual Indexing Reagent 22	<b>N704</b>	<b>S517</b>
Dual Indexing Reagent 23	<b>N705</b>	<b>S517</b>
Dual Indexing Reagent 24	<b>N706</b>	<b>S517</b>

# 96 Dual indexes for MicroPlex Kit v3 - Sets I – IV (C05010004 – C05010007)

Dual indexing reagents are pre-dispensed in 4 different barcoded index plates. Each plate contains one-fourth of the possible dual index combination. Each well of the plate contains sufficient volume for a single use.

Each well of the 96 Dual indexes for MicroPlex Kit v3 contains a unique combination of i7 and i5 index primers carrying the 8-nt dual index sequences shown in Table 2. The dual index combination at each well position is indicated by the column (i7) and row (i5) labels on the plate maps (Figure 1).



**Figure 6.** Combination of i7 and i5 indexes in the 96 Dual indexes for MicroPlex Kit v3, Sets I-IV

## Low-Throughput Applications

The 96 Dual indexes for MicroPlex v3 are designed for high-throughput applications; therefore, the experiment should be designed to pool and sequence the full set of 96 libraries using the entire plate if dual indexes. If indexes from the entire plate are not used at the same time, it is critical to follow the instructions below to avoid cross contamination:

- After removing indexing reagents of choice, cover any pierced or used index wells with scientific tape (e.g., VWR, Cat. No. 89097-920, General-Purpose Laboratory Labeling Tape, 0.5”).
- Thoroughly wipe the seal with 70% ethanol and allow it to dry completely.
- Replace the plastic lid, return the index plate to its sleeve and store at  $-20^{\circ}\text{C}$ .
- The index plate should not be frozen and thawed more than four times.

# Related products

Product	Cat. No.
iDeal ChIP-seq kit for Transcription Factors	C01010055
Auto iDeal ChIP-seq Kit for Transcription Factors	C01010172
iDeal ChIP-Seq Kit for Histones	C01010051
Auto iDeal ChIP-seq Kit for Histones	C01010171
True MicroChIP Kit	C01010130
Auto True MicroChIP Kit	C01010140
iDeal FFPE Kit	C01010190
Universal Plant ChIP-seq Kit	C01010152
Auto Universal Plant ChIP-seq kit	C01010153
MicroPlex Library Preparation Kit v3 x48 rxns	C05010001
MicroPlex Library Preparation Kit v3 x96 rxns	C05010002
24 Dual indexes for MicroPlex Kit v3	C05010003
96 Dual indexes for MicroPlex Kit v3 – Set I	C05010004
96 Dual indexes for MicroPlex Kit v3 – Set II	C05010005
96 Dual indexes for MicroPlex Kit v3 – Set III	C05010006
96 Dual indexes for MicroPlex Kit v3 – Set IV	C05010007
Bioruptor Pico	B01060010
IP-Star Compact Automated System	B03000002

# Technical support

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For technical support contact [custsupport@diagenode.com](mailto:custsupport@diagenode.com)

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