

## I. Introduction

DNA HT Dual Index kits are designed for use with ThruPLEX® and PicoPLEX® library preparation kits 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. The indexed PCR primers are supplied pre-dispensed in four different barcoded index plates, each of which contains one-fourth of the possible dual index combinations (Cat. Nos. R400660–R400663); or in a set of 24 individual tubes, each containing a different dual index combination (Cat. No. R400664).

- Each well of a dual index plate (96N Sets A–D) contains sufficient volume for a single use.
- Each dual index tube (24N) contains sufficient volume for up to two uses.

## II. List of Components

Store all components at –20°C.

**Table 1. DNA HT Dual Index kits.**

Product Name	Cat. No.	Dual index plate	Conc.	Volume per well or tube
DNA HT Dual Index Kit - 96N Set A	R400660	96N Set A	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set B	R400661	96N Set B	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set C	R400662	96N Set C	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set D	R400663	96N Set D	5 µM	5 µl
DNA HT Dual Index Kit - 24N*	R400664	—	5 µM	10 µl

\*Contains 24 different dual index primer combinations in individual tubes labeled as shown in Table 2.

**Table 2. DNA HT Dual Index - 24N (Cat. No. R400664) tube labels.**

N1 - DNA HT Dual Index	N13 - DNA HT Dual Index
N2 - DNA HT Dual Index	N14 - DNA HT Dual Index
N3 - DNA HT Dual Index	N15 - DNA HT Dual Index
N4 - DNA HT Dual Index	N16 - DNA HT Dual Index
N5 - DNA HT Dual Index	N17 - DNA HT Dual Index
N6 - DNA HT Dual Index	N18 - DNA HT Dual Index
N7 - DNA HT Dual Index	N19 - DNA HT Dual Index
N8 - DNA HT Dual Index	N20 - DNA HT Dual Index
N9 - DNA HT Dual Index	N21 - DNA HT Dual Index
N10 - DNA HT Dual Index	N22 - DNA HT Dual Index
N11 - DNA HT Dual Index	N23 - DNA HT Dual Index
N12 - DNA HT Dual Index	N24 - DNA HT Dual Index

### III. Index Sequences

The DNA HT Dual Indexes are 8-nt long and employ the Illumina Nextera XT v2 i5 and i7 dual index sequences. MS-Excel files (XLSX) containing a full list of these indexes and corresponding plate maps can be downloaded from [takarabio.com](http://takarabio.com).

- [DNA HT Dual Index Kit - 96N Set A Indexes and Plate Map](#)
- [DNA HT Dual Index Kit - 96N Set B Indexes and Plate Map](#)
- [DNA HT Dual Index Kit - 96N Set C Indexes and Plate Map](#)
- [DNA HT Dual Index Kit - 96N Set D Indexes and Plate Map](#)
- [DNA HT Dual Index Kit - 24N Indexes](#)

**IMPORTANT:** When using the indexed PCR primers provided in the DNA HT Dual Index kits, make sure to follow the instructions in the user manual that is specific for the kit you are using them with (Section IV.D).

### IV. General Considerations

#### A. Best Practices

Prior to use:

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

**IMPORTANT:** The index plate should not be frozen and thawed more than four times.

#### B. Multiplexing and Index Pooling

It is very important to select appropriate single indexes that are unique and meet the Illumina-recommended compatibility requirements. For low-plex (duplex to 12-plex) pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (Illumina, Publication Document #1000000041074 v05).

#### C. Plate Handling Instructions for Low-Throughput Applications

The DNA HT Dual Index plates are designed for high-throughput applications; therefore, the experiment should be designed to pool and sequence the full set of 96 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:

1. 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").
2. Thoroughly wipe the seal with 70% ethanol and allow it to dry completely.
3. Replace the plastic lid, return the index plate to its sleeve and store at  $-20^{\circ}\text{C}$ .

#### D. Product Compatibility

The DNA HT Dual Index kits are designed for use with the NGS library preparation kits listed below. Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the DNA HT Dual Index kits.

- PicoPLEX Gold Single Cell DNA-Seq Kit (Cat. Nos. R300669, R300670 & R300698)
- ThruPLEX DNA-Seq Kit (Cat. Nos. R400674, R400675, R400676 & R400677)
- ThruPLEX Plasma-Seq Kit (Cat. Nos. R400679, R400680, R400681 & R400682)
- ThruPLEX Tag-Seq kits (Cat. Nos. R400584, R400585 & R400586)

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This document has been reviewed and approved by the Quality Department.