

I. Introduction

DNA unique 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 "IDT for Illumina TruSeq® DNA" 8-nt long index sequences and offer a total of 96 dual indexes for multiplexing of up to 96 samples. The indexed PCR primers are supplied pre-dispensed in four different sets of 24 individual tubes, each of which contains one-fourth of the possible dual index combinations (Cat. Nos. R400665–R400668). Each dual index tube (24U Sets A–D) contains sufficient volume for up to two uses.

II. List of Components

- Store all components at –20°C.
- Each kit contains 24 different dual-index primer combinations in individual tubes.
- Each kit supports 48 reactions.

Table 1. DNA Dual Index kits.

Product Name	Cat. No.	UDIs	Volume/tube	Conc.
DNA Unique Dual Index Kit - 24U Set A	R400665	U1–U24	10 µl	5 µM
DNA Unique Dual Index Kit - 24U Set B	R400666	U25–U48	10 µl	5 µM
DNA Unique Dual Index Kit - 24U Set C	R400667	U49–U72	10 µl	5 µM
DNA Unique Dual Index Kit - 24U Set D	R400668	U73–U96	10 µl	5 µM

III. Index Sequences

The DNA unique dual indexes are 8-nt long and employ the "IDT for Illumina TruSeq DNA" i5 and i7 dual index sequences. MS-Excel files (XLSX) containing a full list of these indexes can be downloaded from takarabio.com.

- [DNA Unique Dual Index Kit - 24U Set A Indexes](#)
- [DNA Unique Dual Index Kit - 24U Set B Indexes](#)
- [DNA Unique Dual Index Kit - 24U Set C Indexes](#)
- [DNA Unique Dual Index Kit - 24U Set D Indexes](#)

IMPORTANT: When using the indexed PCR primers provided in the DNA unique 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.C).

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 pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (Illumina, Publication Document #1000000041074 v05).

C. Product Compatibility

The DNA unique 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 unique 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 & 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.