

# NEXTFLEX<sup>®</sup> Unique Dual Index Barcodes

(Compatible with Illumina<sup>®</sup> platforms)

KIT CONTAINS: 384 OR 1,536 BARCODES | 768 OR 3,072 RXNS

USER MANUAL FOR :  
#NOVA-534100,-01, -02,-03, -04

# NEXTFLEX® Unique Dual Index Barcodes (Set of 1536)

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This product is for research use only.

Not for use in diagnostic procedures.

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# GENERAL INFORMATION

## Product Overview

The NEXTFLEX® Unique Dual Index Barcodes are designed to prepare multiplexed single and paired-end genomic DNA libraries for sequencing using Illumina® platforms. The index and flow cell binding sequences are contained within the NEXTFLEX® Unique Dual Index Barcodes and attached to the sample insert during adapter ligation. Sample pooling with NEXTFLEX® Unique Dual Index Barcodes allows the user to multiplex up to 1536 samples.

Uniquely dual-indexed libraries are libraries prepared with adapters containing two ten base indexes: Index 1 (P7 Index) adjacent to the P7 strand, and Index 2 (P5 Index) adjacent to the P5 strand. None of the indexes found on any given NEXTFLEX® Unique Dual Index Barcode are used throughout the entire set of 1536, which prevents mis-assigned reads from appearing in final data sets. The set of 1536 UDI barcodes are not compatible for use with other NEXTFLEX® Unique Dual Index Barcodes (Set of up to 384).

Each lot of the NEXTFLEX® Unique Dual Index Barcodes is functionally validated and tested for index purity by sequencing.

## Kit Contents, Storage & Shelf Life

The NEXTFLEX® Unique Dual Index Barcodes Kits contains 384 or 1536 uniquely dual-indexed barcoded DNA adapters plated column-wise in a 96-well plate, for a total of 16 plates. See Appendix B for barcode plate configuration. It is recommended that plates and NEXTFLEX® Primer Mix (50 µM) are stored at -20°C. The shelf life of each reagent is at least 1 year when stored properly.

Kit Contents	Cap Color	Amount	Storage Temp.
NEXTFLEX® Unique Dual Index Barcodes 1 - 96* (25 µM)	PLATE	5 µL per well	-20°C
NEXTFLEX® Primer Mix 2.0** (50 µM)	GREEN CAP	384 µL per plate	-20°C

\*The Unique Dual Index Barcodes are supplied in duplex form. Do not heat the adapters above room temperature.

\*\* The Primer Mix 2.0 is only intended for use with the NEXTFLEX® Rapid DNA-Seq Kit 2.0 or NEXTFLEX® Rapid XP DNA-Seq Kit; if the primer mix 2.0 is to be used with any other DNA-Seq kits of choice, additional dilutions may be required. For additional guidance, please inquire at [NGS@revvity.com](mailto:NGS@revvity.com). The kit will be composed of 16 plates, packaged in 16 separate individual boxes. Each box contains a Primer Mix 2.0 vial at the amount indicated above.

## Warnings and Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at [NGS@revvity.com](mailto:NGS@revvity.com).

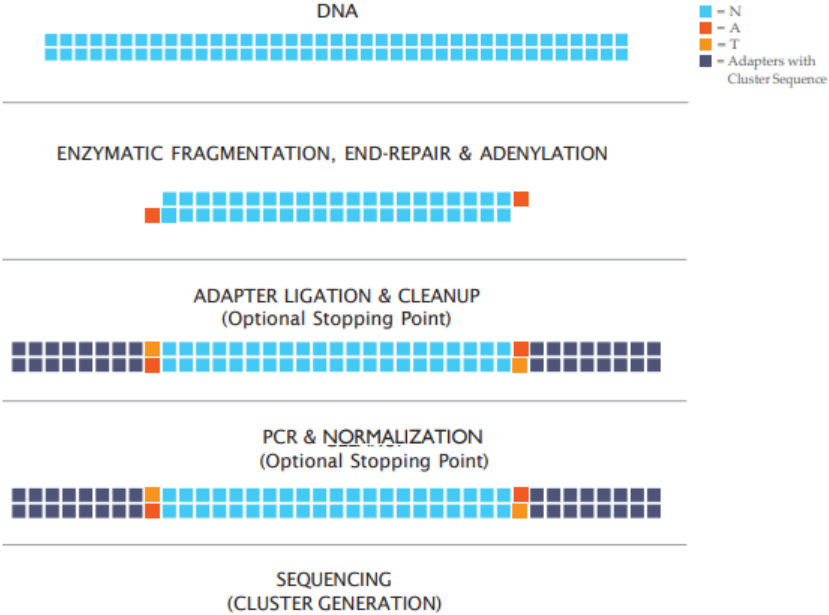
- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTFLEX® Unique Dual Index Barcodes above room temperature.
- Once plate has thawed, spin for one minute before use. This is to ensure all liquid settles to the bottom of the plate.
- The plate seal is intended to be pierced. Do not peel the plate seal from the plate, doing so can easily lead to cross-contamination. Additional thermal heat seals may be applied upon one another to re-seal plate.
- Before use, carefully mix adapters by pipetting up and down several times using a multi-channel pipette with barrier tip. NEVER mix plates by vortexing. Placing a plate on a vortexer to mix samples or barcodes has been proven to result in cross-contamination, even if the plate appears to be securely sealed.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- The NEXTFLEX® Primer Mix 2.0 is only compatible with the NEXTFLEX® Rapid DNA-Seq Kit 2.0 and NEXTFLEX® Rapid XP v2 DNA-Seq Kit at the concentration it is supplied in. If the NEXTFLEX® Unique Dual Index Barcodes are used with other NEXTFLEX® kits, the Primer Mix 2.0 must be diluted prior to use. Make sure to verify that the Primer Mix concentration is compatible at its stock concentration or dilute with nuclease-free water prior to use. The NEXTFLEX® Primer Mix 2.0 should always be used with the NEXTFLEX® Unique Dual Index Barcodes for PCR amplification even if alternative primers are supplied with your kit of choice. Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index. Primer Mix included with the NEXTFLEX® library prep kits should always be used instead of the Primer Mixes that come with the NEXTFLEX barcodes. For additional information, please contact [NGS@revvity.com](mailto:NGS@revvity.com)

## Revision History

Version	Date	Description
V19.10	October 2019	Product Launch
V20.03	March 2020	Change to shelf life. No change to kit material or content.
V21.01	December 2020	Inclusion of part numbers for 384 barcode sets
V23.10	October 2023	Rebrand to Revvity

# SAMPLE PREP PROTOCOL

## NEXTFLEX® Rapid XP v2 DNA-Seq Sample Preparation Flow Chart



# NEXTFLEX® Rapid DNA-Seq 2.0 Sample Preparation Flow Chart



# APPENDIX A

## Oligonucleotide Sequences

NEXTFLEX®	Sequence (5' → 3')
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® UDI Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX <sup>1</sup> ACACTCTTCCCTACACGACGCTCTCCGATCTGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX <sup>2</sup> ATCTCGATGCCGCTCTTCTGCTTG

XXXXXXXX<sup>1</sup> denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

XXXXXXXX<sup>2</sup> denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

The complete index sequences can be found at

[https://resources.revity.com/pdfs/NEXTFLEX\\_UDI\\_Barcodes\\_Sequences\\_1536\\_Set.xlsx](https://resources.revity.com/pdfs/NEXTFLEX_UDI_Barcodes_Sequences_1536_Set.xlsx)

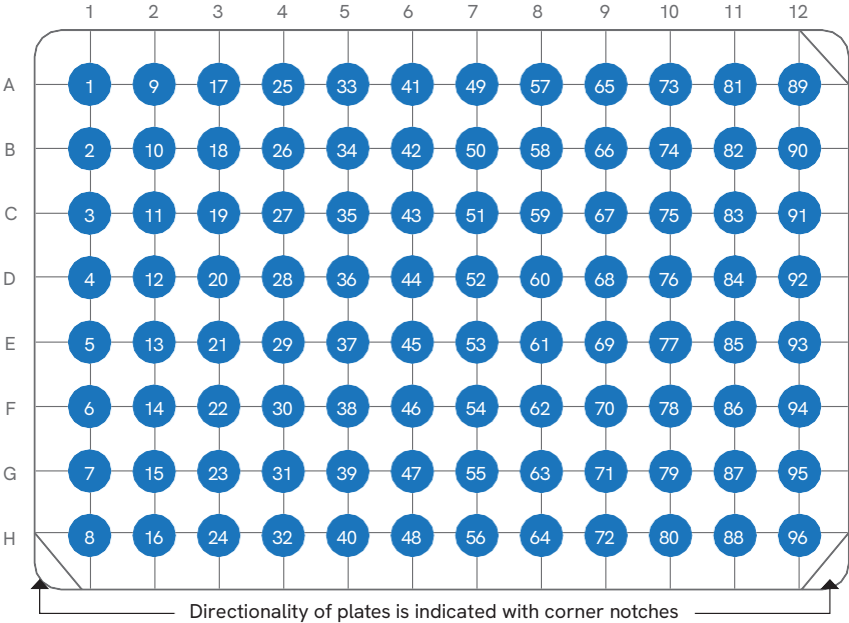
When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column. For additional information, please email [NGS@revity.com](mailto:NGS@revity.com).

# APPENDIX B

## Plate Format

96 Unique Dual Index Barcoded Adapters / Plate; 5  $\mu$ L (2 reaction) / well

Representative Orientation (plate 1 of 16 plates)



NOVA-534100: Barcodes 1-1536

NOVA-534101: Barcodes 1-384

NOVA-534102: Barcodes 385-768

NOVA-534103: Barcodes 769-1152

NOVA-534104: Barcodes 1153-1536





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