NEXTFLEX® UDI-UMI Barcodes

(Compatible with Illumina® & Element® platforms)

KIT CONTAINS: 8, 24, 48, or 96 Barcodes | 8, 24, 48, or 96 RXNS

USER MANUAL FOR:
#NOVA-734100, -01, -02,-03

NEXTFLEX® UDI-UMI Barcodes

This product is for research use only.

Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

Unique Dual Indexes (UDIs) and Unique Molecular Identifiers (UMIs) are nucleotide sequences or "barcodes" that are incorporated during library preparation for Next-Generation Sequencing (NGS) and provide several advantages.

Unique Dual Indexes (UDIs):
10 base pair UDIs are incorporated on both ends of an NGS library molecule, enabling researchers to sequence multiple samples in parallel, which is often referred as multiplexing. The presence of two indices minimizes the effects of index hopping, a phenomenon where a read is assigned to the wrong index as consequence of errors that appear during sequencing process.

Unique molecular identifiers (UMIs):
A single UMI is incorporated on each library. Revvity's 9 bp UMI provides thousands of combinations to uniquely tag each molecule in a sample library. The presence of UMI allows differentiation of PCR duplicates (which contain same UMI sequence) from true copy number (each copy contains a different UMI sequence). By incorporating individual barcodes on each original DNA fragment, variant alleles present in the original sample (true variants) can be distinguished from errors introduced during library preparation, target enrichment, or sequencing. Any identified errors can be removed by bioinformatics methods before final data analysis.

The incorporation of full-length UDI and UMI sequences by ligation instead of PCR reduces the possibility of introducing errors and allows taking advantage of these features in PCR-free workflows.

Kit Contents, Storage & Shelf Life

The NEXTFLEX® UDI-UMI Barcode Kits contains 8, 24, 48, or 96 uniquely dual-indexed UMI Barcodes plated column-wise in a 96-well plate. See Appendix B for barcode plate configurations. It is recommended that plates and NEXTFLEX® Primer Mix 2.0 (50 µM) are stored at -20°C. The shelf life of each reagent is at least 1 year when stored properly.

Note: NEXTFLEX® library preps only require 2.5 µL of each UDI-UMI in the ligation reaction. Other protocols may require different volumes.

Kit Contents	Cap Color	Amount	Storage Temp.
NEXTFLEX® UDI-UMI Barcodes 1 - 96* (15 µM)	PLATE	5 µL per well	-20°C
NEXTFLEX® Primer Mix 2.0** (50 µM)	GREEN CAP	16 µL / 48 µL / 96 µL / 192 µL	-20°C

**The UDI-UMI Barcodes are supplied in duplex form. Do not heat the Barcodes 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 library prep kits, dilutions may be required. For additional guidance, please inquire at https://www.revvity.com/contact-us/technical-support*

Please refer to Appendix B for the configuration of each plate.

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 https://www.revvity.com/contact-us/technical-support and choose Next Gen Sequencing as the category.

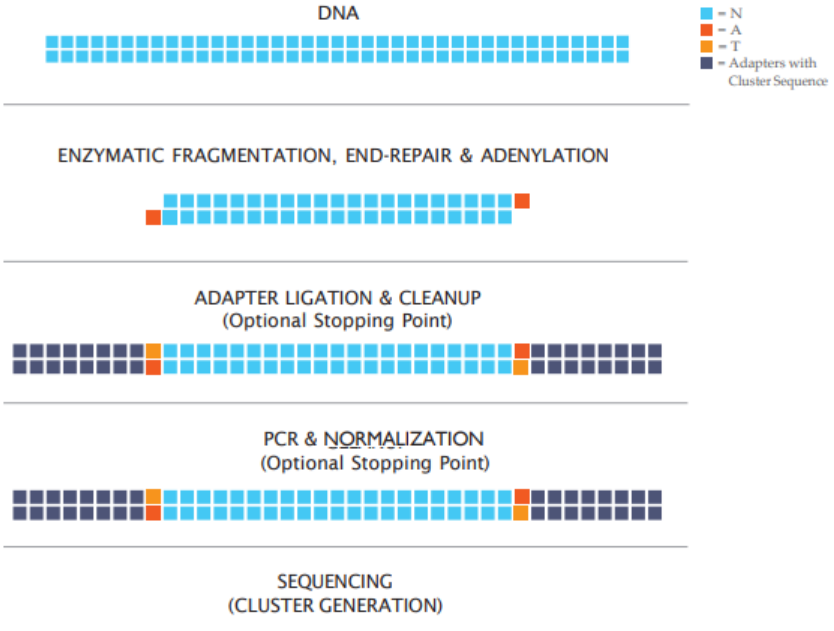
- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTFLEX® UDI-UMI Barcodes above room temperature.
- Once plate has thawed, spin for one minute before use. This is to ensure all 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 Barcodes 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 at. If the NEXTFLEX® UDI-UMI 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® UDI-UMI 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. For additional information, please contact https://www.revvity.com/contact-us/technical-support.

Revision History

Version	Date	Description
V24.07	July 2024	Product Launch
V25.05	May 2025	Quality Assessment Addition

SAMPLE PREP PROTOCOL

NEXTFLEX® Rapid XP v2 DNA-Seq Flow Chart



Note: No barcode dilution required

NEXTFLEX® Rapid DNA-Seq 2.0 Flow Chart

Adapter Dilution Recommendations for NEXTFLEX Rapid DNA-seq 2.0:

Input DNA	Desired Adapter Concentration	Adapter Dilution Required
1 ng	0.3 µM	1 / 50
10 ng	0.6 µM	1 / 25
100 ng	6.25 µM	1 / 2.5
250 ng	15 µM	None
500 ng	15 µM	None
1 µg	15 µM	None

NEXTFLEX® Rapid Directional RNA-Seq 2.0 Flow Chart

FRAGMENTATION USING POLY (A) ENRICHED, rRNA DEPLETED, or TOTAL RNA

FIRST STRAND SYNTHESIS

SECOND STRAND SYNTHESIS

BEAD CLEANUP (Optional Stopping Point)

ADD 'A'

ADD ADAPTERS

LIGATION

BEAD CLEANUP (Optional Stopping Point)

BEAD CLEANUP

NEXTFLEX® Rapid Directional RNA-Seq 2.0 Dilution

Total RNA or Previously Enriched/Depleted RNA		
Previously Poly (A) enriched RNA, Previously rRNA depleted RNA, Total RNA, or FFPE Total RNA	Desired Adapter Concentration	Adapter Dilution Required
1 ng	0.3125 µM	1/50
10 ng	1.56 µM	1/10
50 ng	3.125 µM	1/5
100 ng	6.25 µM	1/2.5

Total RNA		
Total RNA enriched using NEXTFLEX Poly(A) Beads 2.0	Desired Adapter Concentration	Adapter Dilution Required
10 ng	0.104 µM	1/145
100 ng	0.3125 µM	1/50
1,000 ng	1.56 µM	1/10
5,000 ng	6.25 µM	1/2.5

Total RNA		
Total RNA depleted using NEXTFLEX RiboNaut rRNA Depletion Kit (H/M/R)	Desired Adapter Concentration	Adapter Dilution Required
5 ng	0.104 µM	1/145
100 ng	0.3125 µM	1/50
1,000 ng	3.125 µM	1/5

FFPE Total RNA		
FFPE Total RNA depleted using NEXTFLEX® RiboNaut rRNA Depletion Kit (H/M/R)	Desired Adapter Concentration	Adapter Dilution Required
5 ng	0.104 µM	1/145
50 ng	0.104 µM	1/145

QUALITY STATEMENT

Each lot of NEXFLEX Barcodes undergoes strict QC analysis prior to release. Each component must pass rigorous controls standards, and then afterwards the lot is functionally validated by construction and sequencing of NEXTFLEX Rapid DNA-Seq kit 2.0 libraries on Illumina sequencing platforms. The parameters used to release each lot include, among others:

- Reads associated to each index
- % Barcode Purity
- % i5 matching sequence
- % i7 matching sequence

DNA input for quality check are unique sequence-specific amplicons.

APPENDIX A

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5′ → 3′)
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® UDI-UMI Barcodes	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]ACACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX[2]NNNNNNNNN[3]ATCTCGTATGCCGTCTTCTGCTTG

XXXXXXXX[1] denotes the P5 index region of the barcode. The index sequences contained in each barcode are listed at the link provided below.

XXXXXXXX[2] denotes the P7 index region of the barcode. The index sequences contained in each Barcode are listed at the link provided below.

NNNNNNNNN[3] denotes the UMI region of the Barcode.

For a digital copy of indices, please visit our website or this address: https://www.revvity.com/content/nextflex-udi-umi-barcodes-sequences-96-set, or contact us at https://www.revvity.com/contact-us/technical-support and choose the “Next Gen Sequencing” category.

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 contact us at https://www.revvity.com/contact-us/technical-support.

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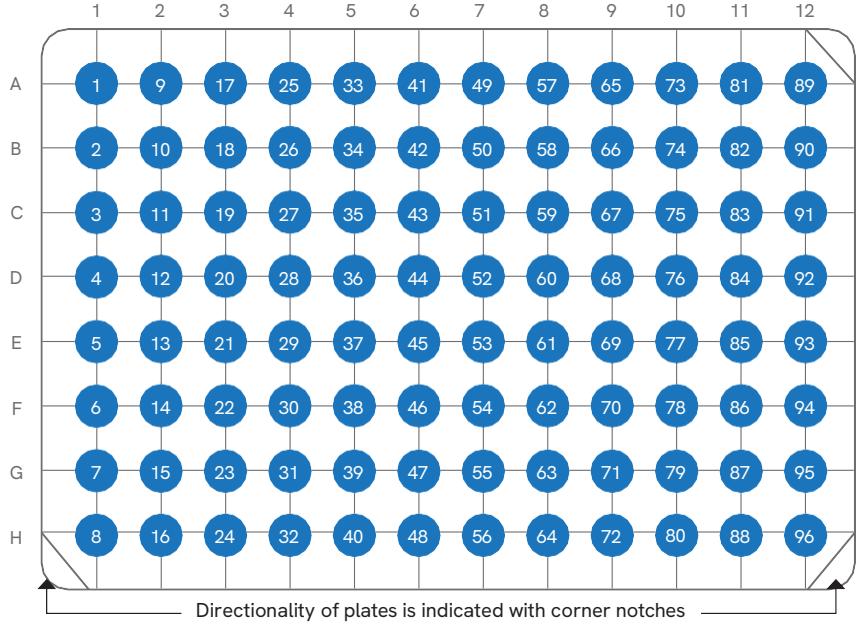
APPENDIX B

Plate Format

96 UDI-UMI Barcodes / Plate; 5 µL / well

Plate Orientation:



NOVA-734100: Contains only Barcodes 1-8 listed in column 1

NOVA-734101: Contains only Barcodes 1-24 listed in columns 1-3

NOVA-734102: Contains only Barcodes 1-48 listed in columns 1-6

NOVA-734103: Contains Barcodes 1-96

APPENDIX C

UMI Analysis Guide

Please refer to this link for the UDI-UMI Analysis guide:

https://resources.revvity.com/pdfs/Revvity_UMI_Analysis_Guide.pdf

www.revvity.com

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