

# NEXTFLEX<sup>®</sup> UDI-UMI Barcodes

(Compatible with Illumina<sup>®</sup> & Element<sup>®</sup>  
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

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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

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.revivity.com/contact-us/technical-support> and choose Next Gen Sequencing as the category.

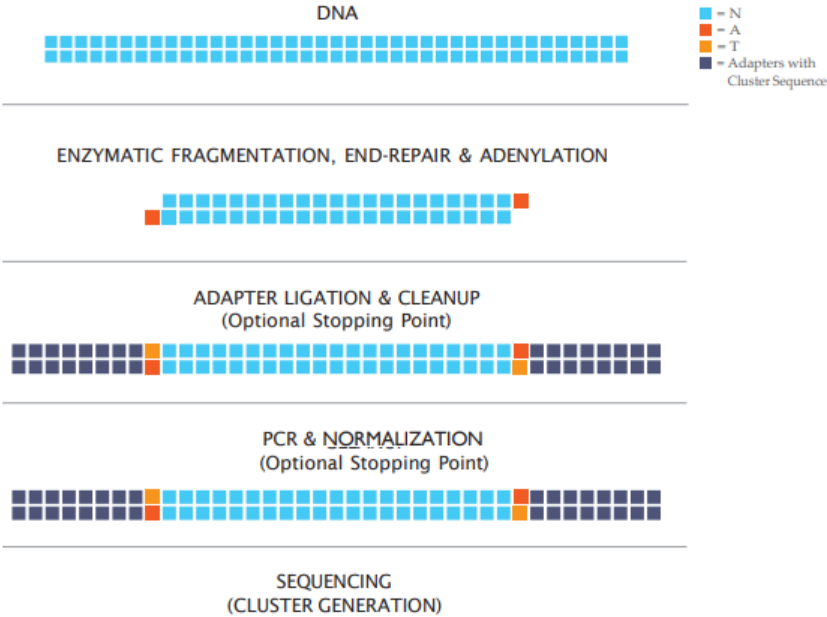
- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTFLEX® UDI-UMI Barcodes above room temperature.
- Once plate has thawed, spin for one minute before use. This is to ensure all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

## Revision History

Version	Date	Description
V24.07	July 2024	Product Launch

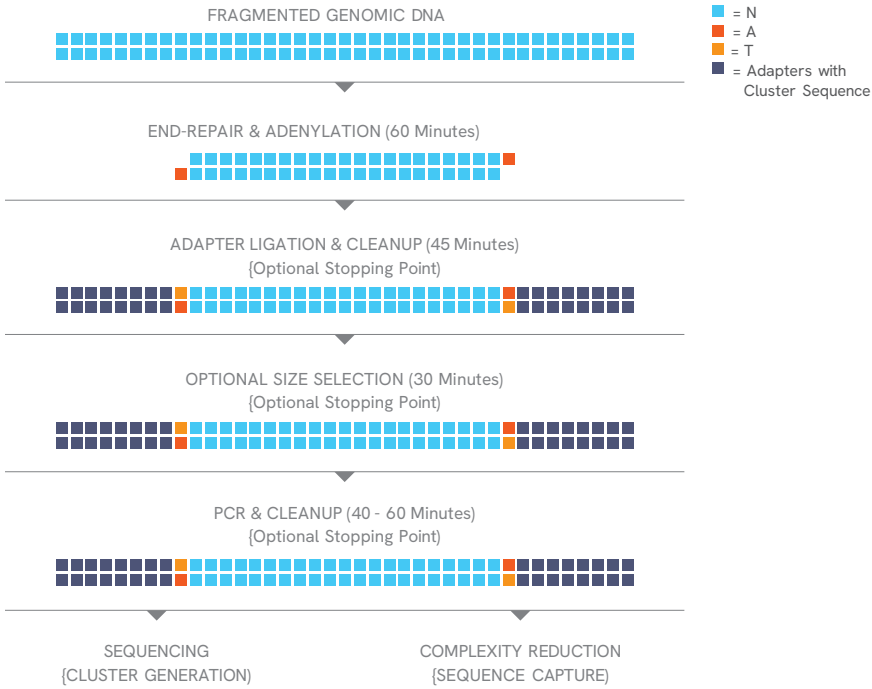
# SAMPLE PREP PROTOCOL

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



Note: No barcode dilution required

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



## Adapter Dilution Recommendations for NEXTFLEX Rapid DNA-seq 2.0:

Input DNA	Desired Adapter Concentration	Adapter Dilution Required
1 ng	0.3 $\mu$ M	1 / 50
10 ng	0.6 $\mu$ M	1 / 25
100 ng	6.25 $\mu$ M	1 / 2.5
250 ng	15 $\mu$ M	None
500 ng	15 $\mu$ M	None
1 $\mu$ g	15 $\mu$ M	None

# NEXTFLEX® Rapid Directional RNA-Seq 2.0 Sample Preparation 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 UDI-UMI Barcode Dilution recommendation:

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



# APPENDIX A

## Oligonucleotide Sequences

NEXTFLEX®	Sequence (5' → 3')
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATAACGAGAT
NEXTFLEX® UDI-UMI Barcodes	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX <sup>1</sup> ACACTCTTCCCTAC ACGACGCTCTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX <sup>2</sup> NNNNNNNN <sup>3</sup> A TCTCGTATGCCGTCTTCTGCTTG

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

XXXXXXXX<sup>2</sup> denotes the P7 index region of the barcode. The index sequences contained in each Barcode are listed at the link provided below.

NNNNNNNN<sup>3</sup> denotes the UMI region of the Barcode.

The complete index sequences list can be found at [https://resources.revity.com/pdfs/NEXTFLEX\\_UDI-UMI\\_Barcodes\\_Sequences\\_96.xlsx](https://resources.revity.com/pdfs/NEXTFLEX_UDI-UMI_Barcodes_Sequences_96.xlsx)

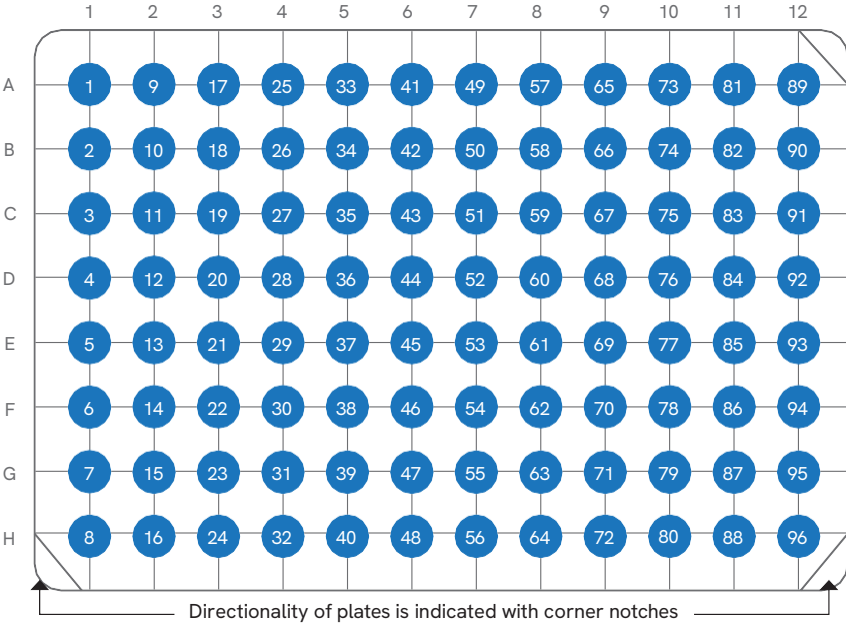
When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column. For additional information, please contact us at <https://www.revity.com/contact-us/technical-support>.

# APPENDIX B

## Plate Format

96 UDI-UMI Barcodes / Plate; 5  $\mu$ L / 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.revivity.com/pdfs/Revvity\\_UMI\\_Analysis\\_Guide.pdf](https://resources.revivity.com/pdfs/Revvity_UMI_Analysis_Guide.pdf)



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