NEXTFLEX® Rapid Directional RNA-Seq Automation Kit 2.0

(For Illumina® & Element® Platforms)

NOVA-5198-53

NEXTFLEX® Rapid Directional RNA-Seq Automation Kit 2.0

This product is for research use only.

Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

Automation of NGS library preparation on the Revvity Sciclone® G3 NGSx workstation reduces sample tracking errors and sample-to-sample variability while dramatically increasing throughput. Samples are processed in 96-well PCR plates, and the number of samples to be processed (1 to 12 columns of 8 samples each) is selected at the start of each run. Pre-set tip-tracking utilities written into the Maestro applications guide the instrument to pick up appropriate numbers of tips and refill/replace tip boxes as needed. Inheco temperature blocks installed on the Sciclone® deck allow for appropriate 4°C storage of reagents and controlled incubation temperatures for enzymatic steps. Reaction mixes are pre-arrayed prior to sample addition to eliminate incubation time variability between samples. The easy-to-follow user interfaces guide the reagent and deck setup process and prompt the user for any necessary interventions.

The NEXTFLEX® Rapid Directional RNA-Seq Automation kit 2.0 is designed to prepare directional, strand-specific RNA libraries from total RNA if used in conjunction with the NEXTFLEX® Poly(A) Beads 2.0 (10 ng - 5 µg) or NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) (5 ng - 1µg) for sequencing using Illumina® and Element® sequencers. The workflow is broken into three separate applications and can be completed in approximately 9-10 hours.

Provided Reagents

Contents, Storage, and Shelf Life

The NEXTFLEX® Rapid Directional RNA-Seq Automation kit 2.0 contains enough material to prepare 96 RNA samples for sequencing on an Illumina® and Element® instrument if used in conjunction with the NEXTFLEX® Poly(A) Beads 2.0 or RiboNaut™ rRNA depletion kit from 10 ng - 5 µg to enrich for mRNA or total RNA (excluding rRNA) from RNA samples of 5 ng – 1 µg total RNA input and NEXTFLEX® adapters. The shelf life of all reagents is at least 12 months when stored properly. The Nuclease-free Water and Resuspension Buffer can be stored at room temperature. The NEXTFLEX® Cleanup Beads XP should be stored at 4°C, and all other components should be stored at -20°C.

Kit Contents	Amount
BROWN CAP	
NEXTFLEX® RNA-Seq Fragmentation Buffer Mix 2.0	760 µL
RED CAP	
NEXTFLEX® RNA-Seq Directional First Strand Synthesis Buffer Mix 2.0	558 µL
NEXTFLEX® RNA-Seq Rapid Reverse Transcriptase 2.0	140 µL
BLUE CAP	
NEXTFLEX® RNA-Seq Directional Second Strand Synthesis Mix 2.0	(2) 1300 µL
CLEAR CAP	
NEXTFLEX® RNA-Seq End Repair & Adenylation Buffer Mix 2.0	(2) 883 µL
NEXTFLEX® RNA-Seq End Repair & Adenylation Enzyme 2.0	353 µL
PURPLE CAP	
NEXTFLEX® RNA-Seq Ligase Buffer Mix 2.0	(4) 1236 µL
NEXTFLEX® RNA-Seq Ligase Enzyme 2.0	334 µL
GREEN CAP	
NEXTFLEX® RNA-Seq PCR Master Mix 2.0	(2) 1314 µL
NEXTFLEX® RNA-Seq Primer Mix 2.0 (50 µM)	211 µL
WHITE CAP BOTTLE	
Nuclease-free Water	25 mL
Resuspension Buffer	20 mL
NEXTFLEX® Cleanup Beads XP	35 mL

Optional Reagents

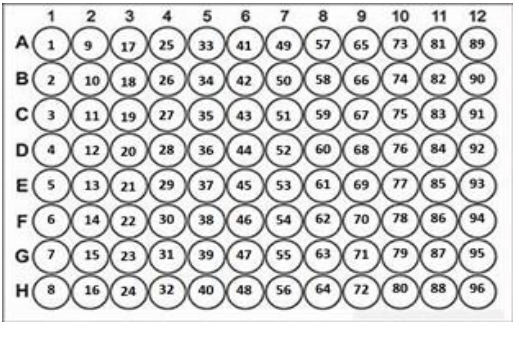
NEXTFLEX Poly(A) Beads 2.0 Automation

Kit Contents	Amount
YELLOW CAP	
NEXTFLEX® Poly (A) Beads 2.0	(2) 1 mL
WHITE CAP BOTTLE	
NEXTFLEX® Poly (A) Washing Buffer 2.0	60 mL
NEXTFLEX® Poly (A) Elution Buffer 2.0	9 mL
NEXTFLEX® Poly (A) Binding Buffer 2.0	21 mL

NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)

Kit Contents	Amount
PINK CAP	
NEXTFLEX® Bait Hybridization Buffer	411 µL
NEXTFLEX® RiboNaut™ Bait Mix	514 µL
BROWN CAP	
NEXTFLEX® Cleanup Beads XP	(2) 1.5 mL
WHITE CAP	
Resuspension Buffer (used as elution buffer)	(2) 1.5 mL
WHITE CAP BOTTLE	
NEXTFLEX® RiboNaut™ Beads	24 mL
NEXTFLEX® RiboNaut™ Bead Wash Buffer	72 mL
NEXTFLEX® Cleanup Buffer	12 mL

NEXTFLEX® Barcoded Adapter Plate



Representative plate layout of NEXTFLEX® RNA-Seq 2.0 UDI Barcodes 1 – 96 (6.25 µM stock plate) for automation. The NEXTFLEX® NGS barcode index is contained within the adapter sequence. For more information on format and customization options for automation, contact us at https://www.revvity.com/contact-us/technical-support.

Sciclone® NGS Hardware, Software, Applications and Consumables

Required Hardware

Part	Vendor/Part Number
Sciclone® NGS Workstation	Revvity
Inheco 384-well plate adapter	NGS Sciclone® accessory CLS 100853
Inheco 96-well adapters (2)	NGS Sciclone® accessory CLS 128372
Inheco 96-well adapter/shaker	NGS Sciclone® accessory CLS 100852
Agencourt® 96 ring magnet	Agencourt® CLS128316
Spacer Assembly for Agencourt® 96 ring magnet	Agencourt® CLS128316

Required Software

- Maestro 6.3 software or later

Provided Maestro Applications

Application Name	NEXTFLEX® Rapid Directional RNA-Seq 2.0 Steps	Approximate Run Time (Including offline Incubations and PCR)
cDNA Synthesis	Optional Poly(A) Enrichment/rRNA Depletion	70 minutes
	RNA Fragmentation	25 minutes
	1st Strand Synthesis	70 minutes
	2nd Strand Synthesis	65 minutes
	Bead Cleanup	45 minutes
Library Prep	3′ Adenylation	45 minutes
	Adapter Ligation	20 minutes
	Bead Cleanups	45 minutes
	PCR Setup and Amplification	30-60 minutes (depending on # of cycles)
Post PCR Cleanup	NEXTFLEX® Bead Cleanups	45-75 minutes (depending on # of cleanups selected)

Required Consumables

Part No.	Vendor	Part	Quantity Needed for 96 samples (including Poly-A enrichment)	Quantity Needed for 96 samples (including RiboNaut™ rRNA Depletion)
6008870	Revvity	Bio-Rad Hard-Shell® 96 Well PCR Plate, Full Skirt	18	16
111426	Revvity	Pipette Tip, 150 µL, Art, Box, 10-96 Sterile Racks	31	33
6008880	Revvity	Deepwell-96 POS, Square 2.0 mL well, Polypropylene, Seahorse	3	3
6008700	Revvity	Reservoir-Deepwell, 12 column, 21mL	4	4
6000030	Revvity	946 Lid-Universal, Robotic friendly, Polystyrene	13	12
6008890	Revvity	Microplate-384 well, Round bottom, Polypropylene, pack of 10	1	1
6008290	Revvity	StorPlate-96V, PP, 96 well, V-bottom, (V), 450µL, 50/box	1	3

Required Materials Not Provided

- Ethanol 100%
- Adhesive PCR Plate Seal (Bio-Rad, Cat # MSB1001)
- Thermal cycler compatible with Bio-Rad Hard-Shell®96 Well Full Skirted PCR Plate
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Microcentrifuge
- 1.5 mL nuclease-free microcentrifuge tubes
- Revvity LabChip® GX/GXII Touch™ instrument or equivalent
- High Sensitivity DNA chips and reagents

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

- Do not use the kit past the expiration date.
- The NEXTFLEX® Directional First Strand Synthesis Buffer Mix 2.0 may appear yellow in color.
- The NEXTFLEX® RNA-Seq 2.0 UDI barcodes (NOVA-51292X series) are intended to be used with the NEXTFLEX® Rapid Directional RNA-Seq 2.0 Kit and are at a 6.25 µM starting concentration. The NEXTFLEX® RNA-Seq Barcodes (NOVA-51291X series) are not compatible with the the NEXTFLEX® Rapid Directional RNA-Seq 2.0 Kit as they are at a concentration of 0.6 µM starting concentration. Most NEXTFLEX adapter barcodes are typically provided at 25 µM, and would require different dilution recommendations if using with the NEXTFLEX® (R) Rapid Directional RNA-Seq 2.0 Kit. Inquire for more details at https://www.revvity.com/contact-us/technical-support.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Ensure that all pipette tips, microcentrifuge tubes, and other consumables are RNase-free.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Vortex and micro-centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.
- Do not remove enzymes from -20°C until immediately before use; return to -20°C immediately after use.
- Do not freeze NEXTFLEX® Cleanup Beads XP.
- Thermal cycling should be performed with a heated lid except where specified.
- Do not heat the NEXTFLEX® adapter barcodes above room temperature.
- For multiplexing options, please use the appropriate NEXTFLEX® adapter barcodes during STEP E: Adapter Ligation.
- RNA sample quality may vary between preparations. High quality RNA should have either an RNA Quality Score (RQS) greater than or equal to 7 or a 28S band that is twice as intense as the 18S band of ribosomal RNA.
- Vortex beads until they are a uniform suspension.
- Allow beads to come to room temperature for 30 minutes prior to use.

Starting Material

The NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0 has been optimized and validated using poly(A) enriched or rRNA depleted RNA (~1 ng – 100 ng). Only 10 ng - 5 µg of total RNA is required if NEXTFLEX Poly(A) beads are used to enrich for mRNA*. The NEXTFLEX® Poly(A) beads have been optimized and validated using 10 ng - 5 µg of total RNA for RNA-seq applications. Only 5 ng - 1 µg of total RNA is required for the NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R).

We recommend examining total RNA integrity using a LabChip® GX/GXII Touch™ instrument or equivalent. High quality total RNA preparations should have an RNA Quality Score (RQS) greater than or equal to 7. Alternatively, total RNA may be run on a 1 - 2% agarose gel and integrity examined by staining with ethidium bromide. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA. Lower amounts of starting material result in higher duplication rates and other changes in sequencing data quality.

*Low RNA inputs may reduce library complexity. To request additional information, email https://www.revvity.com/contact-us/technical-support.

The NEXTFLEX® RNA-Seq 2.0 UDI Barcodes are recommended for use with this kit. These barcodes are at a starting stock concentration of 6.25 µM. If using other NEXTFLEX adapter barcodes, please inquire at https://www.revvity.com/contact-us/technical-support for additional guidance.

Note: Refer to the NEXTFLEX® UDI-UMI Barcode Manual for dilution recommendations

Previously Poly(A) enriched RNA, Previously rRNA depleted RNA, Total RNA, or FFPE Total RNA	Desired Adapter Concentration	Adapter Dilution Required	PCR Cycles
1 ng	0.3125 µM	1/20	13 – 14
10 ng	1.56 µM	1/4	10 – 11
50 ng	3.125 µM	1/2	8 – 9
100 ng	6.25 µM	None	7 – 8
Input RNA: Total RNA enriched using NEXTFLEX® Poly(A) Beads 2.0	**Desired Adapter Concentration**	**Adapter Dilution Required**	**PCR Cycles**
10 ng	0.104 µM	1/60	16 – 17
100 ng	0.3125 µM	1/20	13 – 14
1000 ng	1.56 µM	1/4	9 – 10
5000 ng	6.25 µM	None	7 – 8
Input RNA: Total RNA depleted using NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)	**Desired Adapter Concentration**	**Adapter Dilution Required**	**PCR Cycles**
5 ng	0.104 µM	1/60	16 – 17
100 ng	0.3125 µM	1/20	12 – 13
1000 ng	3.125 µM	1/2	8 – 9

Input RNA: FFPE Total RNA depleted using NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)	Desired Adapter Concentration	Adapter Dilution Required	PCR Cycles
5 ng	0.104 µM	1/60	17 – 20
50 ng	0.104 µM	1/60	14 – 17

APPENDIX

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5′ → 3′)
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® RNA-seq 2.0 UDI Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]AC ACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXX X[2]ATCTCGTATGCCGTCTTCTGCTTG
NEXTFLEX® UDI-UMI Barcodes	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]AC ACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXX X[2]NNNNNNNNN[3]ATCTCGTATGCCGTCTTCTGCTTG

XXXXXXXX[1] denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

XXXXXXXX[2] denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

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

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.

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