NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes

(Compatible with Illumina® and Element® platforms)

KIT CONTAINS : 8, 24, or 96 BARCODES | 16, 48, or 192 RXNS

USER MANUAL FOR :
#NOVA-512920-eval16
#NOVA-512920-eval48
#NOVA-512920
#NOVA-512921
#NOVA-512922
#NOVA-512923

NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes

This product is for research use only.

Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

The NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes are designed to prepare multiplexed single and paired-end cDNA libraries from total RNA, mRNA-enriched RNA or rRNA-depleted RNA for sequencing using Illumina and Element platforms. The index and flow cell binding sequences contained within the NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes attach to the sample insert during adapter ligation. Sample pooling with NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes allows the user to multiplex up to 384 samples when used in conjunction with other available sets of NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes. NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes have been performance-verified to be used in conjunction with the NEXTFLEX Rapid Directional RNA-Seq Kit 2.0.

Uniquely dual-indexed libraries are libraries prepared with adapters containing two eight 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 RNA-Seq 2.0 Unique Dual Index Barcode are used throughout the entire set, which prevents mis-assigned reads from appearing in final data sets.

Each lot of the NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes is functionally validated and tested for index purity by sequencing.

Kit Overview

This NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes Kit contains 8, 24, or 96 uniquely dual- indexed barcoded DNA adapters in plate format for a total of 16, 48, or 192 reactions.

Kit Contents, Storage & Shelf Life

Note: The 16-reaction kit contains UDI barcodes 1-8, the 48-reaction kit contains UDI barcodes 1-24, and the 192-reaction kit contains UDI barcodes 1-96, 97-192, 193-288, or 289-384.

It is recommended that UDI barcodes are stored at -20°C. The shelf life of each reagent is at least 1 year when stored properly.

Kit Contents	Cap Color	Amount (16 rxn / 48 rxn / 192 rxn)	Storage Temp.
NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes* (6.25 µM)	PLATE	5 µL each	-20°C
NEXTFLEX® Primer Mix 2.0** (50 µM)	GREEN CAP	32 µL / 96 µL / 384 µL	-20°C

**These Unique Dual Index Barcodes are supplied in duplex form. Do not heat the adapters above room temperature. All versions of the kit will be plated column- wise (1-8, 9-16, etc.).*

*** The Primer Mix 2.0 is only intended for use with the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0; if the primer mix is to be used with any other RNA-Seq Kits of choice, additional dilutions may be required.*

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 the "Next Gen Sequencing" 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® RNA-Seq 2.0 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.
- NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes have been performance-verified to be used in conjunction with the NEXTFLEX Rapid Directional RNA-Seq Kit 2.0.
- 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 Directional RNA-Seq Kit 2.0 at the concentration it is supplied in. If the NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes are used with other NEXTFLEX® kits, both the Barcodes and Primer Mix 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® RNA-Seq 2.0 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 https://www.revvity.com/contact-us/technical-support.

Version	Date	Description
V20.03	March	Manual Layout
V20.11	November 2020	UDI Index Change
V20.03	March 2020	Manual Layout
V23.10	October 2023	Revvity Rebrand
V25.05	May 2025	Quality Statement Addition

SAMPLE PREP WORKFLOW

NEXTFLEX® Rapid Directional RNA-Seq Kit 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)

PCR

BEAD CLEANUP

UDI Barcoded Adapter Plate Format

Representative plate layout of UDI Barcoded Adapters 1-96, 97-192, 193-288, and 289-834.

NOVA-512920-eval16: Contains only Barcodes 1-8 listed in column 1

NOVA-512920-eval48: Contains only Barcodes 1-24 listed in columns 1-3

1-96 in columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

97-192 in columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

193-288 in columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

289-384 in columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384

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 Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]ACACTCTTTCCCTA CACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX[2]ATCTCGTATG CCGTCTTCTGCTTG

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.

For a digital copy of indices, please visit our website or this address: https://www.revvity.com/content/nextflex-udi-barcodes-sequences-384-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.

Low Level Multiplexing Guidelines

Barcodes 1 and 2, 13 and 14, 25 and 26, 37 and 38, 49 and 50, 61 and 62, 73 and 74, and 85 and 86 are fully color balanced and are suitable to be used in a pool of two samples. When designing low-plexy index pools, always include two libraries barcoded with a set of two unique and fully color balanced barcodes to avoid laser color complexity issues during de-multiplexing. Additional libraries may be safely multiplexed with one set of fully color balanced barcodes in a pool.

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