Illumina library preparation solutions

The foundation to discovery and insights

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Illumina library preparation solutions

Innovations in next-generation sequencing (NGS) solutions continue to improve performance while reducing turnaround time and hands-on time. The Illumina library preparation portfolio offers technology advancements that enable quality, precision, and ease of use for both DNA and RNA sequencing. As the foundation of an end-to-end NGS workflow, these kits are specifically optimized for Illumina instruments and secondary analysis platforms.

Illumina library prep protocols accommodate a range of throughput needs, from lower-throughput protocols for small labs to fully automated workstations for large laboratories or genome centers. Our solutions support a broad range of sample types, from cell culture to fresh tissue, formalin-fixed paraffin-embedded (FFPE) samples, blood, and other challenging sample types.

Illumina library prep for DNA sequencing

The versatile Illumina DNA library preparation portfolio* provides flexibility for examining small, targeted regions or the entire genome. On-bead tagmentation technology enables fragmentation without mechanical shearing to obtain consistent insert sizes and high coverage uniformity. The simplified workflow supports easy volume-based library pooling and minimizes library quantification steps.

Illumina DNA PCR-Free Prep

- Eliminates PCR-induced bias and provides high-performance data for applications that require uncompromised accuracy, such as human whole-genome sequencing
- Delivers libraries in 90 minutes from extracted genomic DNA or just 2.5 hours from raw samples, such as blood, saliva, and dried blood spots
- Supports a broad DNA input range (25-300 ng) and is highly compatible with automation

Illumina DNA Prep[†]

- Offers high performance for various sequencing applications, from large whole genomes to amplicons, plasmids, and microbial species
- Follows a flexible workflow to support a broad DNA input range (1-500 ng), including low-input samples
- Features enhanced library preparation efficiency with integrated DNA extraction protocols for blood, saliva, and dried blood spots

Illumina DNA Prep with Enrichment[‡]

- Supports a broad DNA input range (10-1000 ng) and multiple sample types, including blood, saliva, and FFPE DNA
- Provides a rapid enrichment workflow for targeted resequencing using a single, 90-minute hybridization step
- Enables whole-exome sequencing and advanced study designs in cancer and genetic disease research

* List of DNA library preparation products includes the most current products and is not all inclusive.
† Formerly known as Nextera™ DNA Flex.
‡ Formerly known as Nextera Flex for Enrichment.

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Product	Illumina DNA PCR-Free Prep	Illumina DNA Prep[†]	Illumina DNA Prep with Enrichment[‡]	Nextera XT
Applications and methods	Human whole-genome sequencing	Whole-genome sequencing for large or small genomes	Whole-exome sequencing Targeted resequencing	Small genomes PCR amplicons Plasmids
Mechanism of action	Bead-linked transposome	Bead-linked transposome	Bead-linked transposome and hybrid-capture chemistry	Enzymatic fragmentation
Specialized sample types	Blood Dried blood spots Saliva	Blood Dried blood spots Saliva Bacterial colonies Low-input samples	Blood Saliva FFPE DNA	Bacterial colonies Low-input samples
DNA input amount	25-300 ng	1-500 ng	10-1000 ng 50 ng minimum for complex genomes or FFPE DNA	1 ng
Hands-on time	~45 minutes	1-1.5 hours	~2 hours	15 minutes
Assay time	~1.5 hours	~3-4 hours	~6.5 hours	~5.5 hours
PCR protocol?	No	Yes	Yes	Yes
Library quantification needed?	No	No	No	Yes
Fragmentation included?	Yes–on bead	Yes–on bead	Yes–on bead	Yes
FFPE compatible?	No	Not validated	Yes	No
Automation friendly?	Yes	Yes	Yes	Yes
Compatible panels			Illumina Exome Panel TruSight™ Cancer TruSight Hereditary Cancer TruSight One TruSight One Expanded TruSight Cardio Illumina Custom Enrichment Panel	
Recommended index classes	IDT for Illumina DNA/RNA UD Indexes, Tagmentation	IDT for Illumina DNA/RNA UD Indexes, Tagmentation IDT for Illumina Nextera UD Indexes	IDT for Illumina DNA/RNA UD Indexes, Tagmentation IDT for Illumina Nextera UD Indexes	IDT for Illumina DNA/RNA UD Indexes, Tagmentation IDT for Illumina Nextera UD Indexes
Recommended analysis software	DRAGEN™ Germline TruSight Software Suite	DRAGEN Germline TruSight Software Suite	DRAGEN Enrichment DRAGEN Somatic TruSight Software Suite	SPAdes Genome Assembler BaseSpace™ App
Recommended sequencing systems	NextSeq™ 1000 System NextSeq 2000 System NovaSeq™ 6000 System	iSeq™ 100 System MiniSeq™ System MiSeq™ Series NextSeq Series NovaSeq 6000 System	MiniSeq System MiSeq Series NextSeq Series NovaSeq 6000 System	iSeq 100 System MiniSeq System MiSeq Series NextSeq Series
Link to consumables and equipment list	support.illumina.com/downloads/illumina-dna-pcr-free-consumables-and-equipment.html	support.illumina.com/downloads/illumina-dna-prep-consumables--equipment-list-1000000033564.html	support.illumina.com/downloads/illumina-dna-prep-with-enrichment-consumables-equipment-1000000048602.html	support.illumina.com/downloads/nextera-xt-consumables-equipment-list-1000000006567.html

† Formerly known as Nextera DNA Flex.
‡ Formerly known as Nextera Flex for Enrichment.

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Illumina library prep for RNA sequencing

The Illumina RNA Prep portfolio[§] provides a comprehensive set of solutions to support a variety of applications and sample types. It offers flexibility, scalability, and performance with a rapid, automation-friendly workflow option to prepare sequencing-ready libraries in a single day.

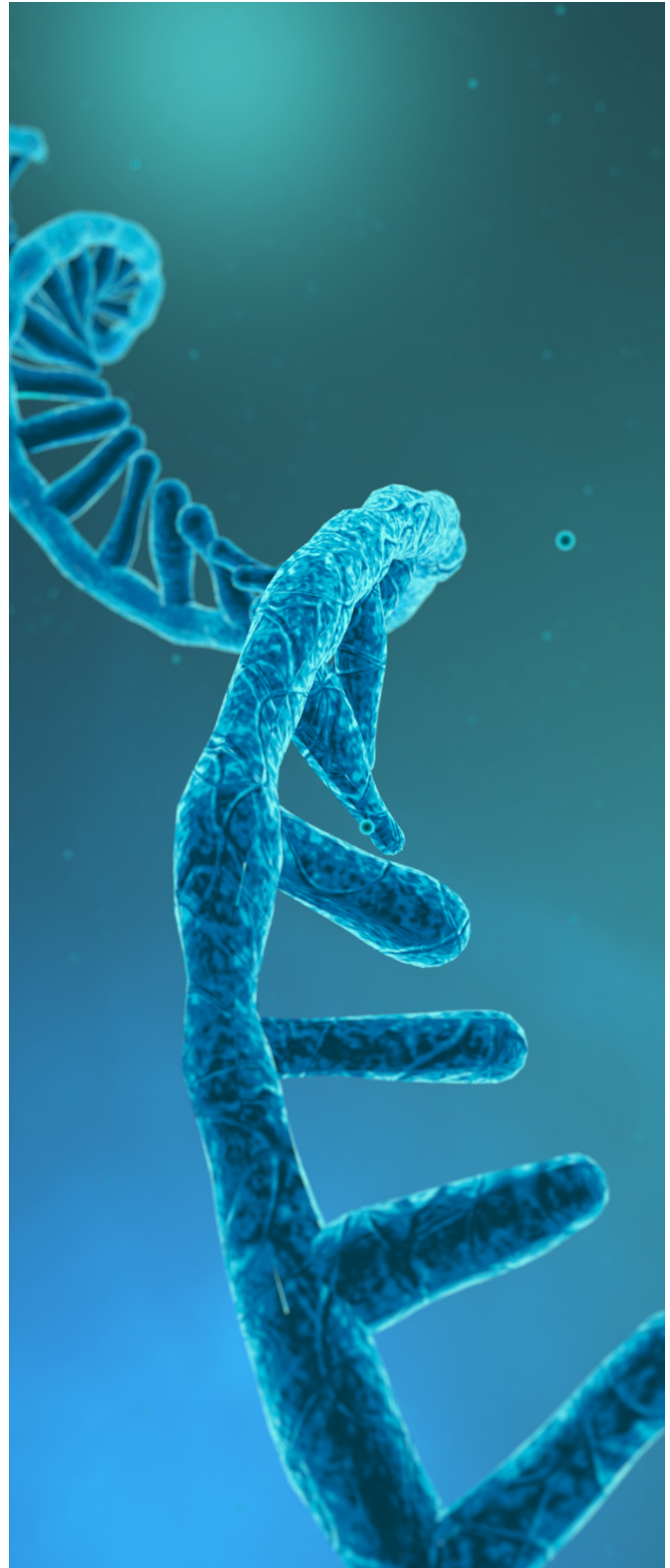## Illumina Stranded Total RNA Prep with Ribo-Zero™ Plus

- Detects coding and noncoding transcripts for whole-transcriptome sequencing
- Supports broad RNA input range (1-1000 ng) and robust performance in low-quality or FFPE samples
- Integrates enzymatic depletion of both ribosomal RNA and globin mRNA in a single, rapid step

Illumina Stranded mRNA Prep

- Delivers accurate, unbiased detection of the protein-coding transcriptome with precise measurement of strand information
- Offers exceptional polyA capture efficiency and coverage uniformity, minimizing sequencing requirements
- Provides wide dynamic range for accurate gene expression profiling using as little as 25 ng high-quality RNA samples

Illumina RNA Prep with Enrichment

- Enriches for targeted sequencing of transcripts of interest, including the RNA exome or respiratory virus detection
- Offers exceptional capture efficiency and coverage uniformity, compatible with low-input or FFPE samples
- Uses simple tagmentation-based library prep and RNA enrichment workflow with minimal hands-on time (< 2 hours)

§ List of RNA library preparation products includes the most current products and is not all inclusive; TruSeq™ products are still available.

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Product	Illumina Stranded Total RNA Prep	Illumina Stranded mRNA Prep	Illumina RNA Prep with Enrichment
Applications and methods	Whole-transcriptome sequencing	mRNA-Seq/Gene expression profiling	RNA exome enrichment mRNA-Seq/Gene expression profiling for low-input or FFPE samples Respiratory virus detection
Mechanism of action	Enzymatic rRNA depletion, ligation-based addition of adapters and indexes	PolyA capture, ligation-based addition of adapters and indexes	Bead-linked transposome and hybrid-capture chemistry
Detection	Coding and noncoding transcriptome	Coding transcriptome with polyA tail	Targeted coding regions
Strand specificity	Stranded	Stranded	Nonstranded
Specialized sample types	Blood FFPE tissue Low-input samples		Blood FFPE tissue Low-input samples Saliva Nasal swabs
RNA input amount	1-1000 ng standard-quality RNA 10 ng minimum for optimal performance and FFPE samples	25-1000 ng standard-quality RNA	10 ng standard-quality RNA 20 ng RNA for low-quality/ FFPE samples
Hands-on time	< 3 hours	< 3 hours	< 2 hours
Assay time	~7 hours	< 7 hours	< 9 hours
Library quantification needed?	Yes	Yes	Yes
Fragmentation included?	Yes	Yes	Not required
FFPE compatible?	Yes	No	Yes
Automation friendly?	Yes	Yes	Yes
Compatible panels			Illumina Exome Panel Respiratory Virus Oligo Panel v2
Recommended index classes	IDT for Illumina RNA UD Indexes, Ligation	IDT for Illumina RNA UD Indexes, Ligation	IDT for Illumina DNA/RNA UD Indexes, Tagmentation
Recommended analysis software	DRAGEN RNA Pipeline DRAGEN Differential Expression	DRAGEN RNA Pipeline DRAGEN Differential Expression BaseSpace Correlation Engine	DRAGEN RNA Pipeline DRAGEN Differential Expression DRAGEN RNA Pathogen Detection App
Recommended sequencing systems	NextSeq Series NovaSeq 6000 System	NextSeq Series NovaSeq 6000 System	MiniSeq System MiSeq Series NextSeq Series NovaSeq 6000 System
Link to consumables and equipment list	support.illumina.com/downloads/ illumina-stranded-total-rna-consumables-1000000124517.html	support.illumina.com/downloads/ illumina-stranded-mrna-consumables-1000000124520.html	support.illumina.com/ downloads/illumina-rna-prep-consumables-1000000124437.html

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Ordering information

	Product	Catalog no.
DNA library prep kits	Illumina DNA PCR-Free Prep, Tagmentation (24 samples)	20041794
	Illumina DNA PCR-Free Prep, Tagmentation (96 samples)	20041795
	Illumina DNA Prep, (M) Tagmentation (24 samples)	20018704
	Illumina DNA Prep, (M) Tagmentation (96 Samples)	20018705
	Illumina DNA Prep, (S) Tagmentation (16 samples)	20025519
	Illumina DNA Prep, (S) Tagmentation (96 samples)	20025520
	Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)	20025523
	Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)	20025524
	Nextera XT DNA Library Preparation Kit (24 samples)	FC-131-1024
	Nextera XT DNA Library Preparation Kit (96 samples)	FC-131-1096
RNA library prep kits	Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (16 samples)	20040525
	Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (96 samples)	20040529
	Illumina Stranded mRNA Prep, Ligation (16 samples)	20040532
	Illumina Stranded mRNA Prep, Ligation (96 samples)	20040534
	Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples)	20040536
	Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples)	20040537
	Flex Lysis Reagent Kit (96 reactions)	20018706
Indexes	IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20027213
	IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20027214
	IDT for Illumina Nextera DNA Unique Dual Indexes Set C (96 indexes, 96 samples)	20027215
	IDT for Illumina Nextera DNA Unique Dual Indexes Set D (96 indexes, 96 samples)	20027216
	IDT for Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20040553
	IDT for Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20040554
Enrichment panels	Illumina Exome Panel – Enrichment oligos only	20020183
	Respiratory Virus Oligos Panel V2	20044311
	TruSight Cancer – Enrichment oligos only (4 or 8 enrichment reactions)	FC-121-0202
	TruSight Hereditary Cancer – Enrichment oligos only (8 enrichment reactions)	20029551
	TruSight One – Enrichment oligos only (6 enrichment reactions)	20029227
	TruSight One Expanded – Enrichment oligos only (6 enrichment reactions)	20029226
	TruSight Cardio – Enrichment oligos only (8 enrichment reactions)	20029229
	Illumina Custom Enrichment Panel (8 enrichment reactions)	20025371

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