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DATA SHEET

Illumina miRNA Prep

Fast, reliable miRNA and small RNA sequencing library generation

Saves time and reduces sample loss by eliminating the need for gel purification

Improves detection in low-input RNA samples by preventing adapter dimer formation

Optimizes quantification accuracy by reducing biases and background interference

Introduction

MicroRNAs (miRNAs) are small, noncoding RNA molecules that regulate gene expression by inhibiting the translation of mRNA or targeting it for degradation.[1] They play critical roles in cellular processes and development, making them a key research focus for understanding disease mechanisms and developing novel therapeutic strategies.

Next-generation sequencing (NGS) enables high-throughput, precise profiling of miRNA expression, allowing researchers to identify novel miRNAs, quantify their expression levels, and uncover their roles in gene regulation.[2,3] The short length of miRNAs makes them susceptible to biases introduced during isolation and library preparation that can affect quantification accuracy. Limited availability of input RNA can also increase the risk of sequencing artifacts and reduce sensitivity for low-abundance miRNAs.[4,5]

Illumina miRNA Prep provides a simple, cost-effective solution for generating miRNA and small RNA sequencing libraries directly from total RNA or isolated miRNA for virtually any species. Illumina miRNA Prep uses an optimized chemistry that prevents adapter dimers, minimizes biases, and improves quantification of even limited miRNA samples.

About Illumina miRNA Prep

The Illumina miRNA Prep streamlined workflow begins with purified total RNA or isolated miRNA from which miRNA libraries are prepared, followed by sequencing and data analysis (Figure 1).

The optimized adapter ligation, amplification, and cleanup steps allow researchers to move from purified total RNA or isolated miRNA to sequencing-ready miRNA libraries in less than a day (Table 1). Key advantages of Illumina miRNA Prep include:

- **Optimized adapter ligation:** ensures selective capture of mature miRNA during library preparation using specially designed 3′ and 5′ adapters
- **Streamlined bead-based purification:** reduces hands-on time and workflow complexity by eliminating tedious gel-based size selection
- **Targeted prevention of adapter dimers:** preserves sequencing reads for true miRNA molecules using modified oligonucleotides that reduce adapter dimer formation

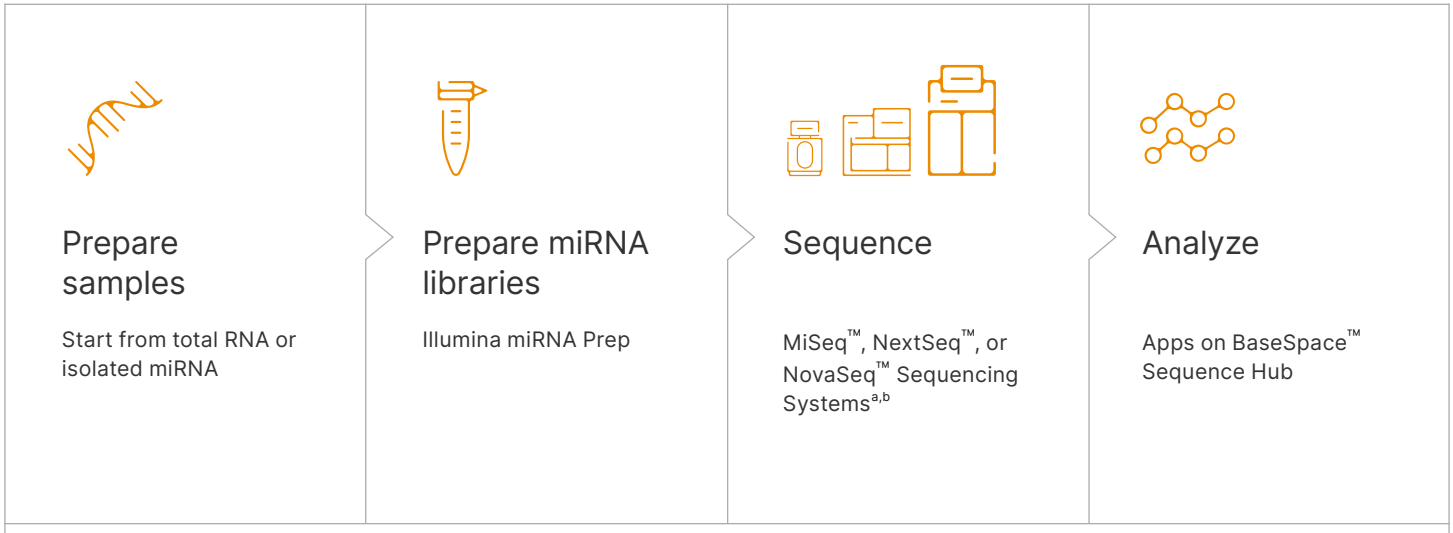

Figure 1: Illumina miRNA Prep workflow
Total RNA or isolated miRNA is used to generate miRNA-specific libraries, which can be sequenced on all Illumina NextSeq, NovaSeq, or MiSeq Systems (Table 1). Illumina BaseSpace Sequence Hub provides software tools for primary and secondary analysis.

a. Includes NextSeq 500, NextSeq 550, NextSeq 1000, NextSeq 2000, NovaSeq 6000, NovaSeq X, NovaSeq X Plus, MiSeq, and MiSeq i100 Systems.
b. Using 384 unique dual indexes.

- **Precise incorporation of Unique Molecular Identifiers (UMIs) during reverse transcription:** enables accurate quantification by tagging each original miRNA molecule to distinguish unique molecules from PCR duplicates
- **Enhanced bias reduction in PCR amplification:** improves detection sensitivity and ensures reliable quantification of low-abundance miRNAs with minimal RNA input through optimized chemistry that promotes uniform PCR amplification

Table 1: Illumina miRNA Prep specifications

Parameter	Illumina miRNA Prep	TruSeq Small RNA
Input type	Total RNA from cells, FFPE tissue, serum/plasma, or fresh-frozen tissue from virtually any species	Optimized using high-quality UHR total RNA[a]
Input required	1–500 ng[b]	1000 ng
No. available indexes	384 UDI	48 single indexes
Supported sequencing systems	Illumina NextSeq, NovaSeq, and MiSeq Systems[c,d]	Illumina MiSeq, NextSeq 500/550 Systems[e]
Workflow time[f]	3 hr hands-on, 7 hr total	~4-5 hr hands-on, ~ 11 hr total, or ~ 1 day total with overnight elution

a. Using RNA from other species, tissues, or qualities may require further optimization.
b. For best results, higher amounts of total RNA are recommended, especially for low-abundance miRNA samples.
c. Includes NextSeq 500, NextSeq 550, NextSeq 1000, NextSeq 2000, NovaSeq 6000, NovaSeq X, NovaSeq X Plus, MiSeq, and MiSeq i100 Systems.
d. Using 384 unique dual indexes.
e. Limited by number of available index sets.
f. Starting from isolated total RNA to library cleanup.

FFPE, formalin-fixed, paraffin-embedded; UHR, universal human reference; UDI, unique dual indexes.

Optimized miRNA isolation

Unlike most cellular RNAs, mature miRNAs have both a 3′ hydroxyl group and a 5′ phosphate group. These features facilitate the ligation of unique adapters that enable amplification of mature miRNAs while minimizing amplification of other types of RNA (Figure 2).

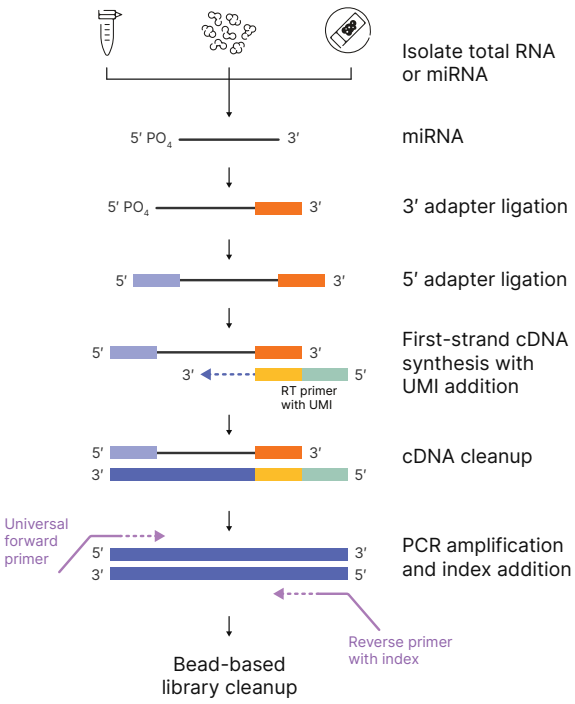

Figure 2: Illumina miRNA Prep chemistry
Adapters are ligated to 5′ and 3′ ends of each miRNA to ensure preferential amplification. Unique molecular identifiers (UMIs) are integrated during the reverse transcription (RT) step, enabling digital quantification and correcting for amplification bias. A rapid, bead-based cleanup replaces gel purification, streamlining the workflow to just seven hours.

Gel-free, bead-based cleanup

Gel-based purification of amplified libraries is labor-intensive, requiring electrophoresis, band excision, and elution, yet it often fails to remove all adapter dimers and contaminating RNA. Illumina miRNA Prep eliminates these steps with a gel-free, bead-based workflow (Figure 3) that improves miRNA-specific isolation while minimizing adapter dimers and unwanted RNA types.

Unbiased quantification

Illumina miRNA Prep improves miRNA quantification by using UMIs to count individual molecules (Figure 2). Traditional library preparation methods often introduce bias during amplification, leading to overrepresentation of certain miRNAs and distortion of expression levels. Because UMIs are incorporated at an early stage, each miRNA molecule is counted only one time, distinguishing true biological signals from artifacts such as adapter dimers (Figure 4).

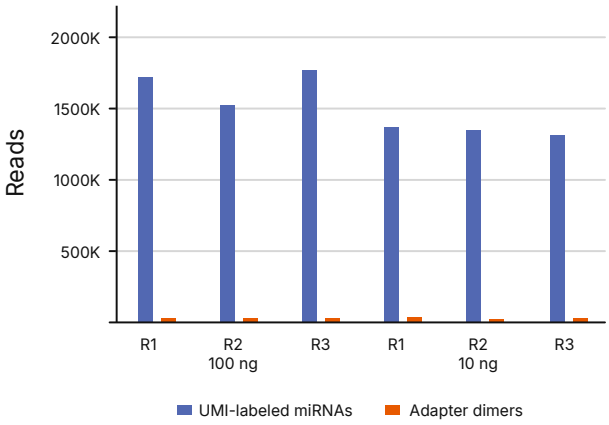

Figure 4: Unbiased miRNA quantification using UMIs
By incorporating unique molecular identifiers (UMIs) early in the workflow, most sequencing reads are mapped to unique miRNAs, minimizing bias from adapter dimers.

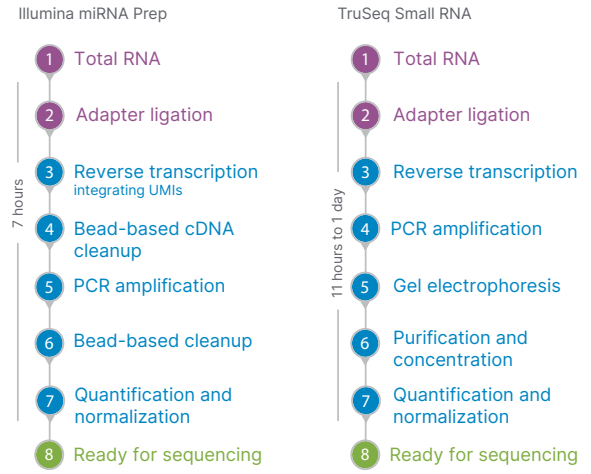

Figure 3: Illumina miRNA Prep and TruSeq Small RNA workflow comparison
By eliminating gel electrophoresis and purification, Illumina miPrep mRNA Prep delivers a faster workflow with reduced hands-on time compared to TruSeq Small RNA. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience.

Maximized sensitivity

miRNAs have emerged as biomarkers for diseases such as cancer and neurodegenerative disorders.[6-8] Exosomes, small extracellular vesicles secreted by cells, also carry miRNAs and protect them from degradation, increasing their stability.[9] Additionally, miRNAs are well preserved in formalin-fixed, paraffin-embedded (FFPE) tissues due to their small size, enabling retrospective studies on archived samples.[10]

Because miRNAs in biofluids and FFPE samples are often present in low abundance, highly sensitive library preparation methods are required for accurate detection. Illumina miRNA Prep addresses this challenge by enabling miRNA profiling from as little as 1 ng of total RNA. This capability is beneficial for studies using biofluids such as serum (Figure 5).

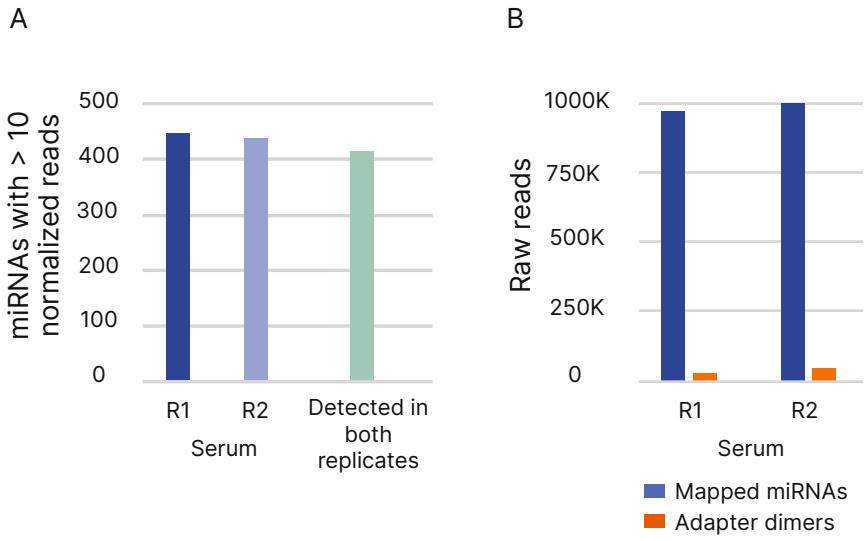

Figure 5: Robust miRNA detection in serum samples

Illumina miRNA Prep provides robust, reproducible miRNA detection in biofluids such as serum. (A) Detection of miRNA from replicate serum samples (R1 and R2). (B) Reads of mapped miRNAs compared to adapter dimers from replicate serum samples.

miRNA analysis simplified

Illumina offers powerful software solutions for analyzing miRNA sequencing data, streamlining the identification and quantification of miRNAs. BaseSpace Sequence Hub and Illumina Connected Analytics (ICA) provide secondary analysis of Illumina miRNA Prep data, including read alignment, mapping, and quantification. The resulting output files can then be used for tertiary analyses such as differential expression. These tools help researchers efficiently process miRNA data, supporting biomarker discovery and expression studies with reliable, user-friendly analysis.

Summary

Illumina miRNA Prep delivers a fast, gel-free workflow for miRNA sequencing library preparation, integrating adapter ligation and UMI tagging to maximize accuracy. The user-friendly workflow supports diverse sample types, from biofluids to FFPE tissue, and enables high-throughput miRNA profiling with minimal hands-on time. The optimized chemistry ensures preferential miRNA amplification while eliminating adapter dimers and contaminating RNA, resulting in cleaner libraries and more accurate quantification. By optimizing workflow efficiency and minimizing artifacts for greater reliability, Illumina miRNA Prep improves the accuracy and impact of miRNA research.

Learn more →

Illumina miRNA Prep

BaseSpace Sequence Hub

Introduction to RNA sequencing

Ordering information

Product	Catalog no.
Illumina miRNA Prep (96 samples)	20145030
Illumina miRNA UD Indexes Set A (96 indexes, 96 samples)	20145031
Illumina miRNA UD Indexes Set B (96 indexes, 96 samples)	20145032
Illumina miRNA UD Indexes Set C (96 indexes, 96 samples)	20145033

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