# RealSeq®-Biofluids

**FOR SMALL RNA DETECTION**

## Highly efficient small RNA detection from biofluids

**Gel-free extracellular small RNA profiling from as little as 50 µl plasma**

## Obtain libraries with more representation of different ncRNA species

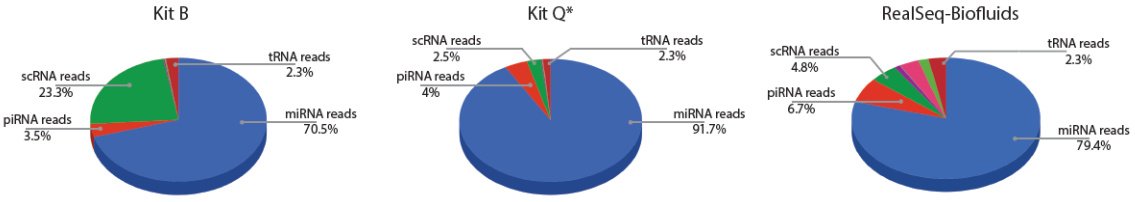


**Figure 1 | The RealSeq-Biofluids kit provides libraries with a higher percentage of reads mapping to different classes of ncRNAs compared to other kits.** Kit Q, according to the manufacturer, is specifically designed to remove reads mapping to HY4 RNA (scRNA) impeding its quantification.

Complete small RNA library preparation kit for Illumina sequencing for RNA samples from biofluids and other sample types with low RNA concentration including cell-free (cf)RNA. Patented technology using a single-adapter and circularization strategy reduces detection bias for accurate small RNA profiling. Size selection reagent and barcoded PCR primers are included.

### Optimized for biofluid samples

- Plasma and other biofluid samples contain extremely low concentrations of cf-miRNAs
- Accurate and sensitive quantification of cf-miRNAs from biofluids requires different reaction conditions compared to tissue samples
- Gel-free detection is a must for reproducible and automatable biomarker discovery pipelines
- RealSeq-Biofluids capitalizes on the accuracy of RealSeq-AC while sensitive enough to allow gel-free detection of cf-miRNAs from biofluids

- **Accurate and sensitive quantification of cf-miRNAs from biofluids**
- **Gel-free detection for reproducibility and use with automated biomarker discovery pipelines**
- **Allows discovery of novel small RNAs from samples with extremely low concentrations**

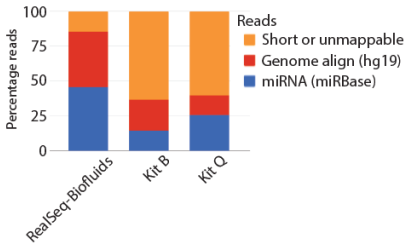


**Figure 2 |** Comparison of read length representation in libraries prepared from plasma samples using RealSeq-Biofluids vs two other kits.

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# RealSeq-Biofluids | Higher detection efficiency vs other kits

## Easily incorporates into typical RNA-seq workflow

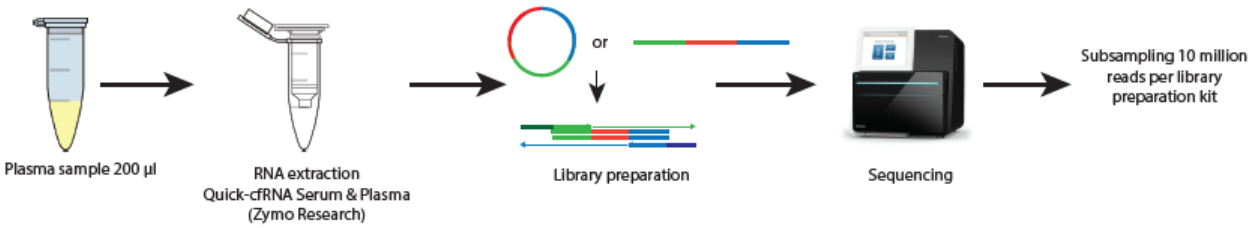


**Figure 3 | A common library preparation workflow.** The same plasma sample was used to prepare sequencing libraries with RealSeq-Biofluids and two other kits. The panels below show sequencing metrics of libraries prepared with each kit.

## Detection of plasma miRNA with RealSeq-Biofluids vs other kits

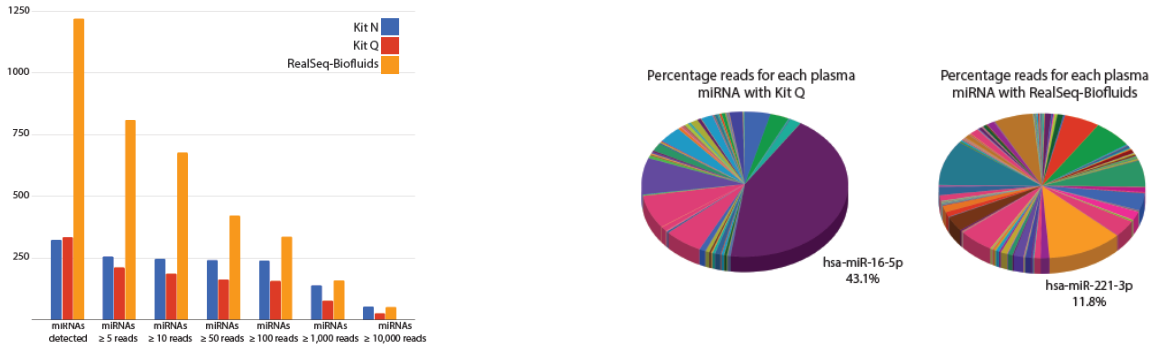


**Figure 4 | Profiling of plasma miRNAs with three different library preparation kits.** 200 µl of plasma sample from a healthy donor was used to extract RNA with Quick-cfRNA Plasma/Serum kit (Zymo research) following manufacturer recommendations. RNA from three extractions was pooled and used to prepare sequencing libraries with the three kits following manufacturer recommendations for gel-free libraries. To normalize for sequencing coverage reads were subsampled to 10 million reads per kit. Sequencing reads were processed as Figures 2-3, except that reads were aligned to a reference that includes all human miRNAs in miRBase 21. The left panel shows the number of miRNAs detected at different coverage for each library preparation kit. The right panel shows the percentage of plasma reads for each miRNA with kits Q and RealSeq-Biofluids.

## The high sensitivity and efficiency of RealSeq technology enables efficient detection of small RNAs from low-concentration samples

- RealSeq-Biofluids allows preparation of gel-free sequencing libraries with an RNA input obtained from only 50 µl of plasma
- RealSeq-Biofluids delivers the highest percentage of usable reads (>15 nt and that align to either miRBase or genome) of the 3 kits tested
- Highly accurate profiling allows the identification of a larger set of cf- miRNAs (Figure 4, left)
- Detection bias inherent in the two-adapter platforms reduces the number of miRNAs identified (Figure 4, left)
- Detection bias also results in the overrepresentation of a few miRNAs that consume the majority of sequencing reads (Figure 4, left)
- scRNAs are overrepresented in libraries prepared with the two-adapter ligation scheme (Figure 1), while they represent only 4% of the reads for RealSeq-Biofluids (single-adapter and circularization)
- RealSeq-Biofluids allows accurate and sensitive quantification of cell-free miRNAs with a gel-free protocol

## Contact us today to get started.

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