



# Comprehensive Evaluation of cfRNA Recovery Efficiency Across Tube Types and Competitors Using the Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit

## Introduction

The Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit is designed to deliver high-quality isolation of cell-free RNAs (cfRNA) ranging from 17 to 1,000 nt, without using phenol or chloroform. This kit is powered by Apostle MiniMax<sup>®</sup> technology which uses magnetic nanoparticles. This solution ensures high recovery efficiency and supports compatibility with a wide range of downstream applications, including next-generation sequencing (NGS), PCR and other molecular assays.

The kit is optimized for samples collected in major blood collection tubes (BCTs), particularly those that preserve RNA integrity during storage, making it ideal for liquid biopsy workflows. By providing reliable cfRNA isolation, the Apostle MiniMax<sup>®</sup> Kit empowers clinical researchers across diverse therapeutic areas such as oncology, cardiovascular, neuroscience research, prenatal and gestation-related studies, virology and public health.

## Objective

To quantify and compare cfRNA recovery and size range performance of the Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit versus a column-based competitor across commonly used BCTs. Performance was evaluated by qPCR amplification plots and  $C_t$  values from a cel-miR-39 spike-in and representative endogenous targets (beta-globin, miR-21, U6 and miR-15).

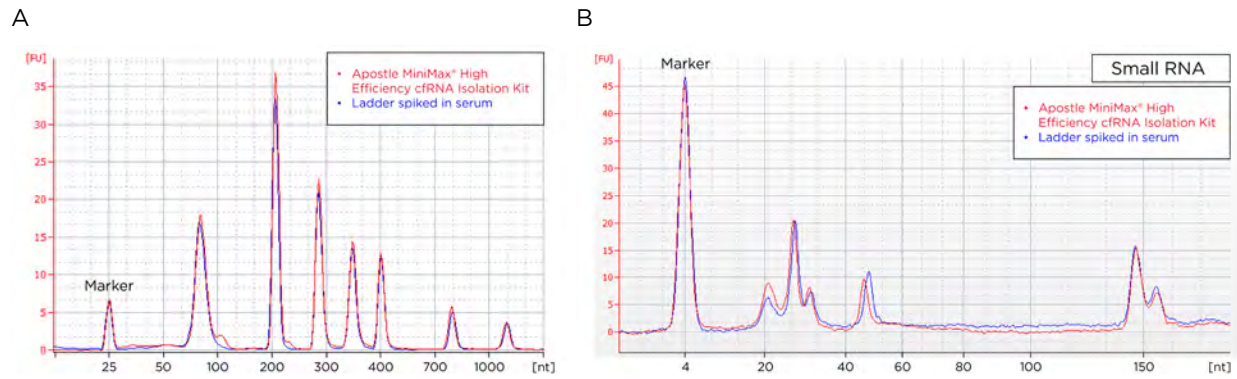
## Results and Discussion

### Performance Evaluation of Apostle MiniMax<sup>®</sup> Kit Using RNA Size Standards

To evaluate the performance of the Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit, RNA and small RNA ladders (Ranging from 17-1,000 nt) were spiked into plasma and serum samples, followed by cfRNA isolation using the kit. The recovered RNA was analyzed using the Agilent Bioanalyzer 2100, and the electropherograms were compared to the original RNA ladder.

**Figure 1A** shows the recovery profile for RNA fragments ranging from 100-1,000 nt, while **Figure 1B** illustrates the recovery of small RNAs between 17-150 nt. In both cases, the red curves (Apostle MiniMax<sup>®</sup> Kit) closely overlap with the blue curves (original ladder), indicating highly efficient recovery across the entire size range. Quantitative analysis demonstrated an RNA recovery efficiency greater than 85% for fragments between 17 and 1,000 nt.

These results confirm that the Apostle MiniMax<sup>®</sup> Kit provides superior cfRNA isolation, ensuring minimal loss of small RNA species and recovering both long and short cfRNA fragments with high efficiency.



**Figure 1:** Recovery profile of RNA (A) and small RNA (B) ladders using Apostle MiniMax® High Efficiency cRNA Isolation Kit.

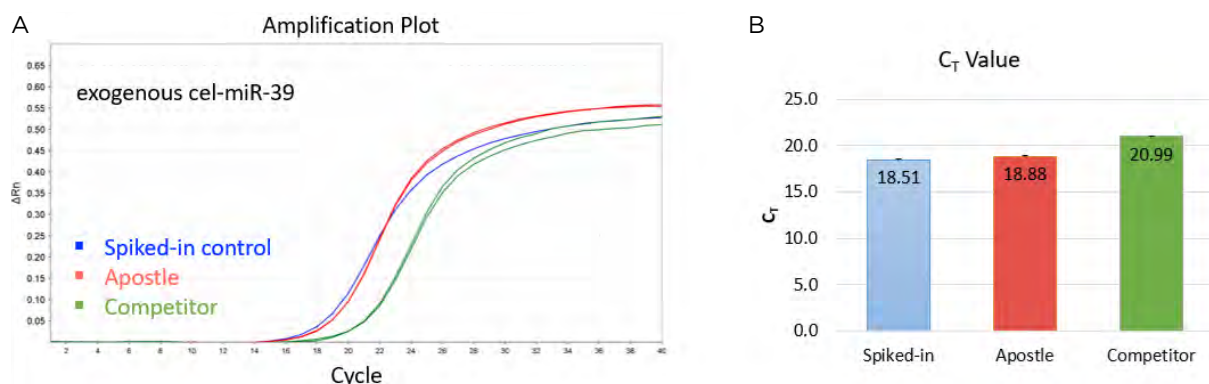
### qPCR and $C_t$ -Value-Based Assessment of cRNA Recovery Efficiency Compared to a Column-Based Competitor

The qPCR amplification plot and  $C_t$  analysis demonstrate a significant difference in cRNA recovery efficiency between the Apostle MiniMax® High Efficiency cRNA Isolation Kit and a major column-based competitor. Plasma samples were spiked with synthetic cel-miR-39 RNA as an exogenous control prior to extraction.

- The amplification plot shows that cRNA recovered using the Apostle MiniMax® Kit (red curve) closely aligns with the spike-in control (blue curve), indicating minimal loss during extraction (**Figure 2A**).
- In contrast, the competitor (green curve) exhibits delayed amplification, reflecting lower recovery efficiency (**Figure 2A**).
- The  $C_t$  values further confirm this trend: spike-in control (18.51), Apostle MiniMax® Kit (18.88), and competitor (20.99) (**Figure 2B**).
- These differences correspond to an 85% recovery rate for the Apostle MiniMax® Kit compared to 18% for the competitor, equating to a 4.3-fold higher RNA recovery using the Apostle MiniMax® Kit (**Figure 2B**).

The results highlight the superior performance of the Apostle MiniMax® High Efficiency cRNA Isolation Kit in recovering cRNA from plasma samples. The close alignment of the Apostle amplification curve with the spike-in control demonstrates that the kit preserves RNA integrity and minimizes template loss during extraction (**Figure 2A**).

The significantly lower  $C_t$  value for Apostle MiniMax® Kit compared to the competitor indicates higher sensitivity and efficiency (**Figure 2**). The amplification is not inhibited, which indicates that the Apostle MiniMax® Kit does not contain contaminants which interfere with enzymatic reactions, making it highly suitable for clinical research workflows. By achieving 4.3 times higher recovery than a major alternative (**Figure 2B**), the Apostle MiniMax® Kit ensures reliable detection of low abundance cRNAs, reducing variability and improving assay sensitivity.



**Figure 2:** (A) qPCR and (B)  $C_t$ -value-based evaluation of cRNA recovery using synthetic cel-miR-39 spike-in compared to a competitor.

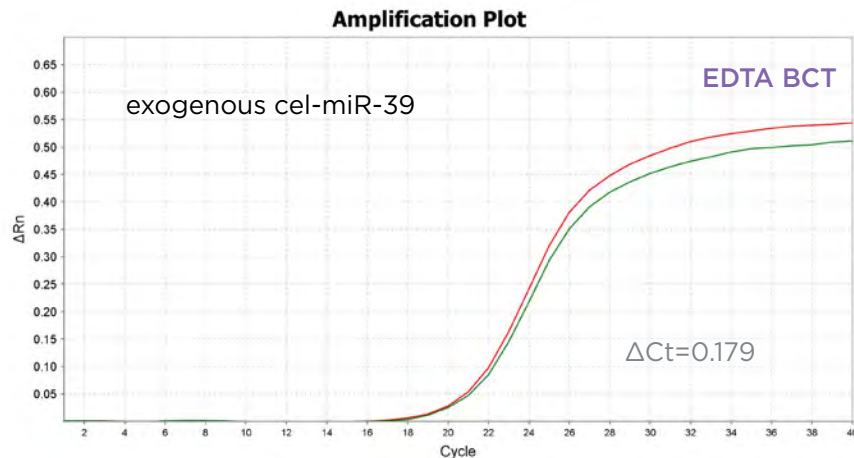
## Comparative Recovery of Exogenous cel-miR-39 Across Different BCT Types Compared to a Major Column-Based Competitor

The qPCR amplification plots demonstrate significant differences in exogenous cel-miR-39 recovery rates across different BCT types. Plasma samples processed using the Apostle MiniMax® High Efficiency cfRNA Isolation Kit (red curves) consistently exhibited earlier amplification (lower  $C_t$  values) compared to the column-based competitor (green curves), indicating higher RNA recovery efficiency. Specifically:

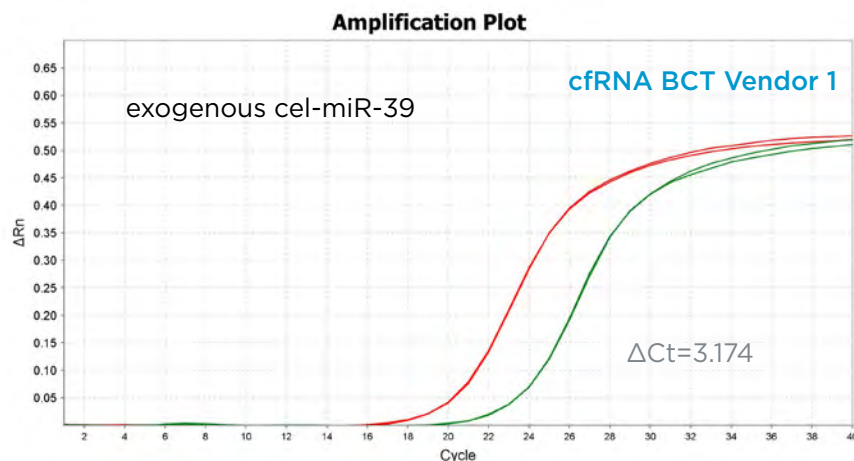
- For EDTA BCT, the  $\Delta C_t$  between the two methods was minimal ( $\Delta C_t = 0.179$ ), suggesting comparable recovery (**Figure 3A**).
- For cfRNA BCT Vendor 1, the  $\Delta C_t$  was 3.174, corresponding to approximately a 9-fold higher recovery with the Apostle MiniMax® Kit (**Figure 3B**).
- For cfRNA BCT Vendor 2, the  $\Delta C_t$  was 11.462, translating to nearly a 2,000-fold higher recovery using the Apostle MiniMax® Kit (**Figure 3C**).

These results highlight substantial variability in cfRNA recovery depending on both the BCT type and the extraction method. The data underscore the critical impact of extraction methodology and tube type on cfRNA yield from plasma. While EDTA tubes showed negligible differences between the two methods, cfRNA-specific BCTs exhibited pronounced disparities. The Apostle MiniMax® High Efficiency cfRNA Isolation Kit demonstrated superior performance, particularly for cfRNA BCT Vendor 2, where recovery was dramatically higher compared to the column-based competitor. This suggests that the Apostle MiniMax® Kit is highly effective in overcoming potential inhibitory factors or stabilizers present in certain cfRNA BCTs, which may hinder column-based extraction efficiency.

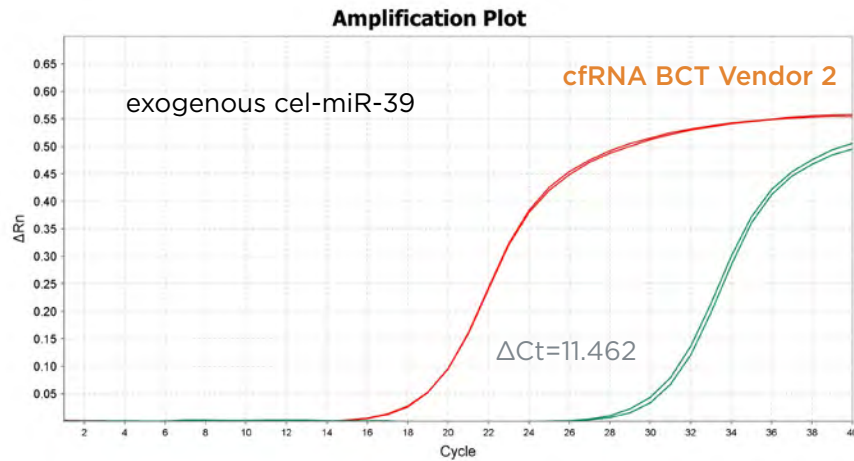
A



B



C



**Figure 3:** Amplification plots comparing exogenous cel-miR-39 recovery from plasma using two extraction methods across different BCT types.

### Quantification of $\beta$ -globin, miR-21, U6, and miR-15 by qPCR Amplification and $C_t$ Values Using Two Extraction Methods Across Different BCT Types

The qPCR amplification plots reveal significant differences in cfRNA recovery efficiency between the Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit and a column-based competitor across three BCT types: Streck cfRNA BCTs (vendor 1), PAXgene BCTs (vendor 2) and K<sub>3</sub>EDTA BCTs.

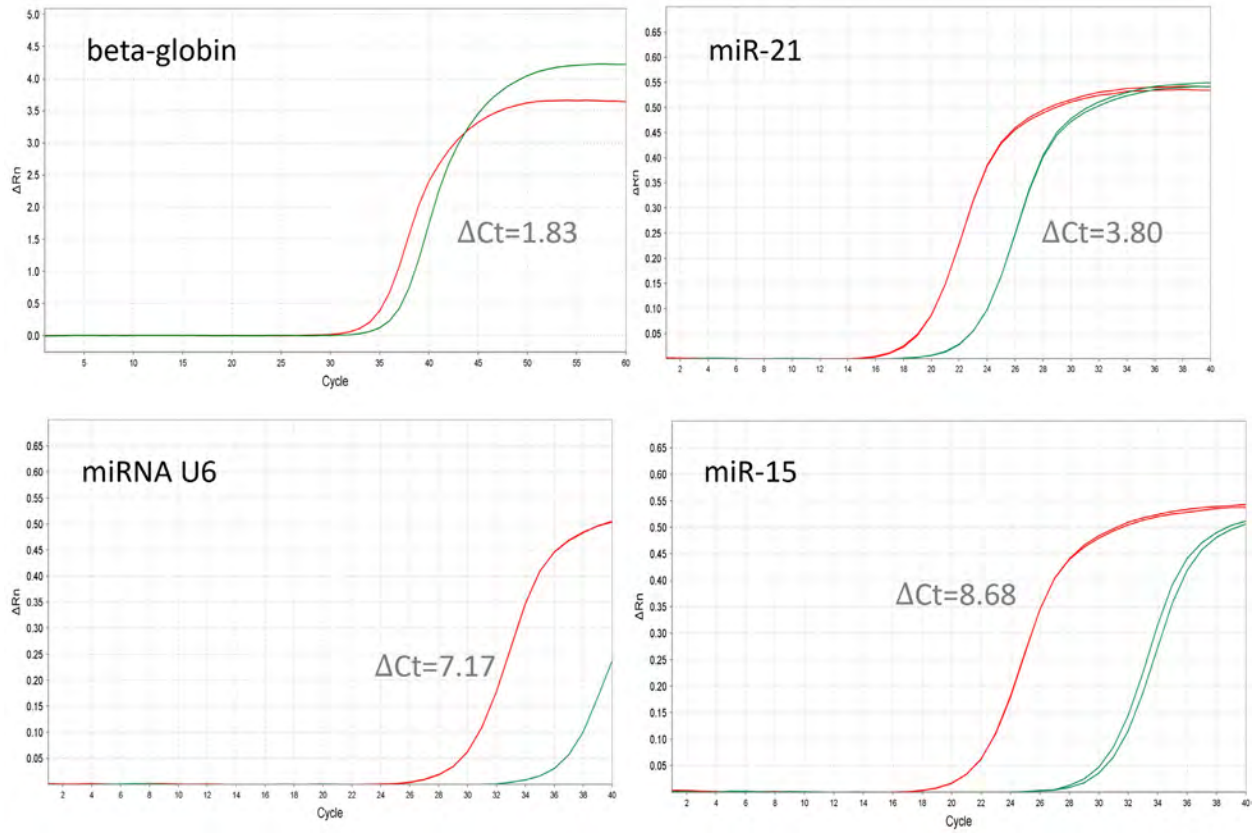
- **Streck cfRNA BCTs (Vendor 1):** The Apostle MiniMax<sup>®</sup> Kit demonstrated markedly better amplification (lower  $C_t$  values) compared to the competitor for all tested RNA targets:  $\beta$ -globin ( $\Delta C_t = 1.83$ ), miR-21 ( $\Delta C_t = 3.80$ ), miRNA U6 ( $\Delta C_t = 7.17$ ), and miR-15 ( $\Delta C_t = 8.68$ ). These differences correspond to 3.5- to 480-fold higher RNA recovery rates with the Apostle MiniMax<sup>®</sup> Kit (**Figure 4A**).
- **PAXgene BCTs (Vendor 2):** Recovery improvements for the Apostle MiniMax<sup>®</sup> Kit were moderate but consistent, with  $\Delta C_t$  values of 1.18 ( $\beta$ -globin), 2.54 (miR-21), 0.36 (U6), and 2.61 (miR-15), translating to 1.6- to 6-fold higher recovery compared to the competitor (**Figure 4B**).
- **EDTA BCTs:** The Apostle MiniMax<sup>®</sup> Kit showed measurable improvements, with  $\Delta C_t$  values of 1.086 ( $\beta$ -globin), 0.186 (miR-21), 1.26 (U6), and 0.368 (miR-15), corresponding to 1.1- to 2.4-fold higher recovery (**Figure 4C**).

Across all tube types, the Apostle MiniMax<sup>®</sup> Kit consistently outperformed the column-based competitor, particularly for small RNA species such as miRNA U6 and miR-15, which are critical for biomarker discovery.

These results highlight the strong performance of the Apostle MiniMax<sup>®</sup> High Efficiency cfRNA Isolation Kit in recovering cfRNA from plasma collected in various BCTs. The most pronounced advantage was observed with Streck cfRNA BCTs, where recovery rates were up to 480 times higher than the column-based competitor, especially for small RNAs. This suggests that the Apostle MiniMax<sup>®</sup> Kit effectively mitigates challenges posed by stabilizers or additives in specialized cfRNA tubes, which may inhibit column-based extraction methods. For PAXgene and EDTA tubes, the Apostle MiniMax<sup>®</sup> Kit still provided measurable improvements.

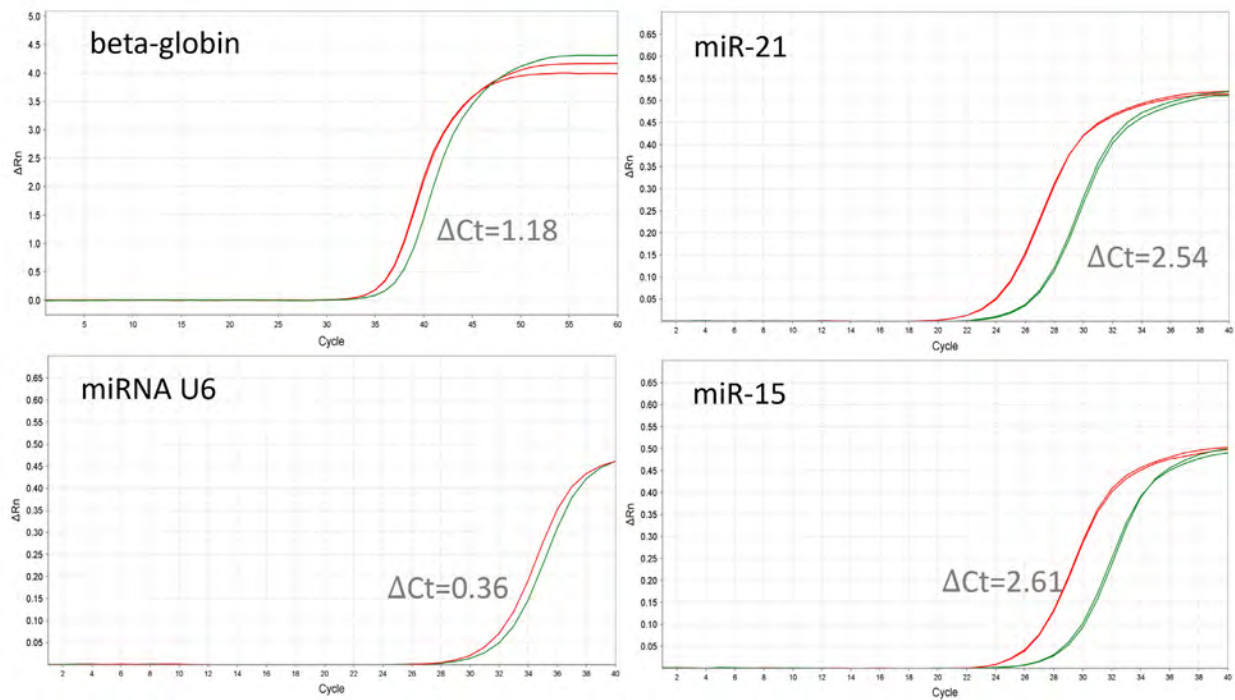
A

**Amplification Plot**



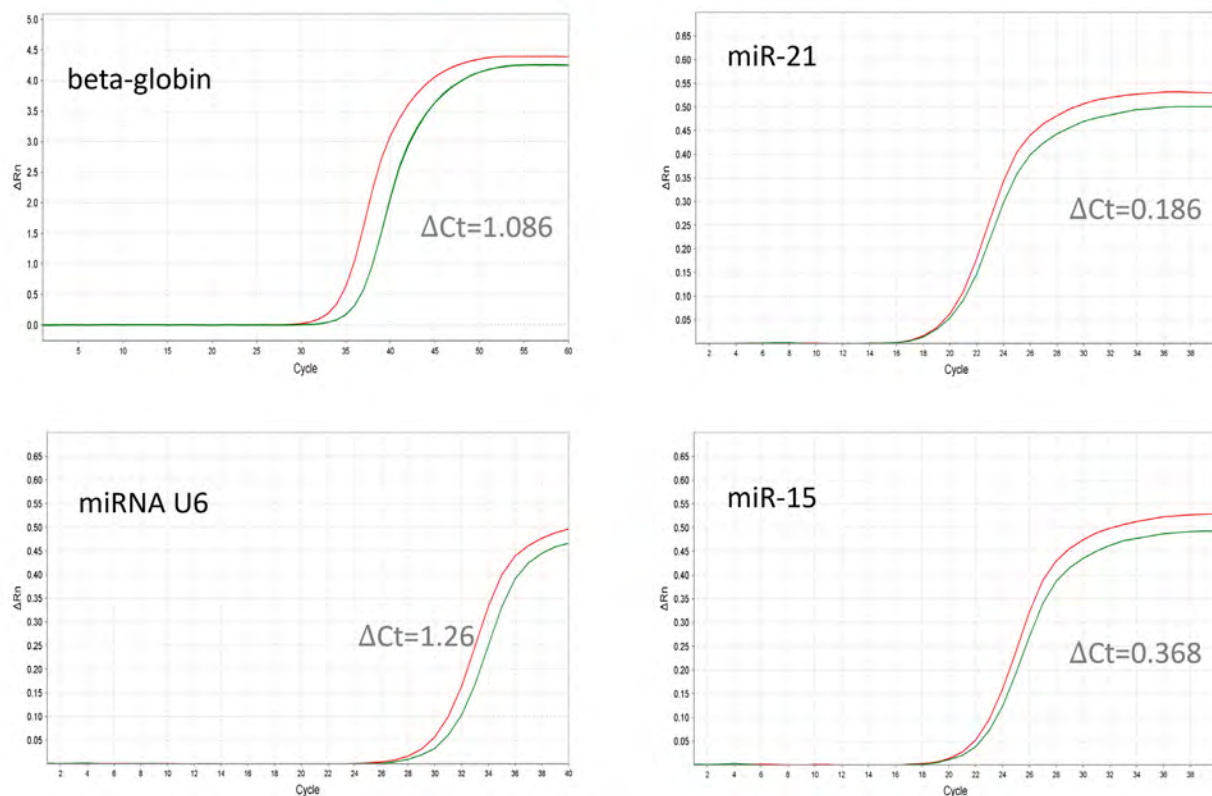
B

**Amplification Plot**



C

### Amplification Plot



**Figure 4:** cfRNA recovery using the Apostle MiniMax® Kit versus a column-based competitor using (A) Streck cfRNA BCTs (Vendor 1) (B) PAXgene BCTs (Vendor 2) and (C) EDTA BCTs.

### Superior cfRNA Recovery Across Multiple BCT Types

The qPCR amplification plots and  $C_t$  values demonstrate clear differences in cfRNA recovery efficiency between the Apostle MiniMax® High Efficiency cfRNA Isolation Kit and a major column-based competitor across three BCT types: EDTA, PAXgene, and Streck cfRNA BCTs.

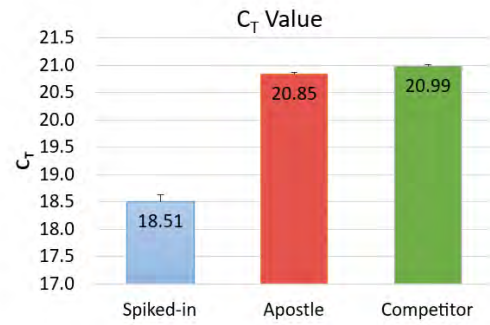
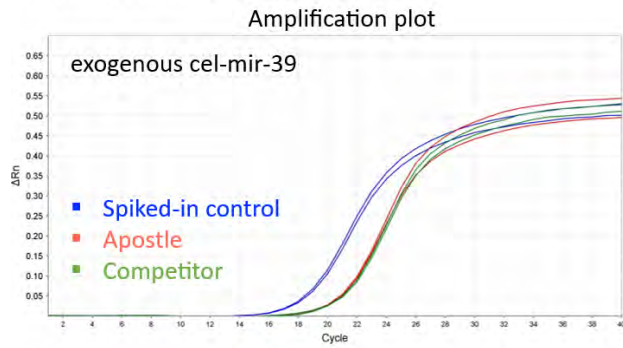
- **EDTA BCTs:** The amplification plot shows that cfRNA recovered using the Apostle MiniMax® Kit (red curve) closely aligns with the spike-in control (blue curve), while the competitor (green curve) amplifies later, indicating lower recovery.  
 $C_t$  values: Spike-in control (18.51), Apostle MiniMax® Kit (20.85), competitor (20.99) (**Figure 5A**).
- **PAXgene BCTs:** The Apostle MiniMax® Kit again outperforms the competitor, with amplification curves closer to the spike-in control.  
 $C_t$  values: Spike-in control (18.51), Apostle MiniMax® Kit (20.22), competitor (23.40) (**Figure 5B**).
- **Streck cfRNA BCTs:** The most pronounced difference is observed here.  
 $C_t$  values: Spike-in control (18.51), Apostle MiniMax® Kit (18.88), competitor (30.34) (**Figure 5C**).

Across all tube types, the Apostle MiniMax® Kit consistently demonstrates superior cfRNA recovery, particularly for Streck cfRNA BCTs. These findings confirm that the Apostle MiniMax® High Efficiency cfRNA Isolation Kit provides robust and reliable cfRNA recovery across diverse pre-analytical conditions. The close alignment of Apostle MiniMax® Kit amplification curves with the spike-in control indicates minimal loss during extraction and absence of inhibitors.

The dramatic performance gap observed with Streck cfRNA BCTs highlights the kit's ability to overcome challenges posed by stabilizers or additives that can hinder column-based extraction methods. Even in standard EDTA and PAXgene tubes, the Apostle MiniMax® Kit consistently delivers higher recovery rates, reducing variability and improving sensitivity for low abundance cfRNA targets.

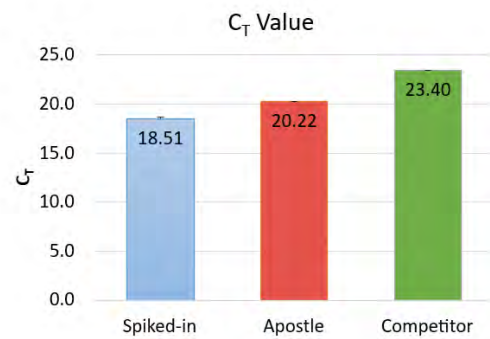
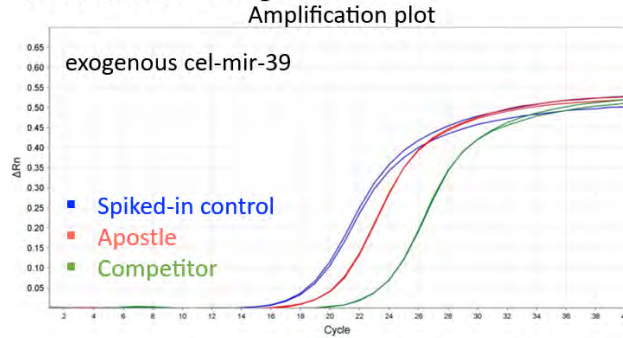
A

Blood collected in K<sub>3</sub>EDTA BCTs

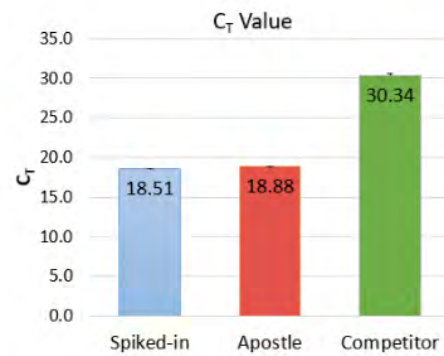
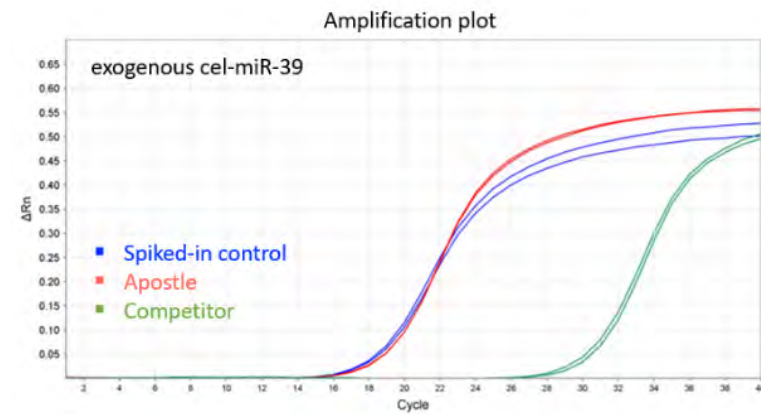


B

Blood collected in PAXgene BCTs



C



**Figure 5:** qPCR-based evaluation of cfRNA recovery from blood collected in (A) EDTA, (B) PAXgene and (C) Streck cfRNA BCTs using Apostle MiniMax® Kit versus a column-based competitor.

## Conclusion

The Apostle MiniMax® High Efficiency cfRNA Isolation Kit consistently demonstrated superior performance in recovering cfRNA across a broad size range (17-1,000 nt) and under diverse pre-analytical conditions. Electropherogram analysis confirmed high recovery efficiency (>85%) for both long and short RNA fragments, while qPCR data revealed up to 4.3-fold higher recovery compared to a major column-based competitor.

Furthermore, the kit maintained RNA integrity and minimized template loss, ensuring reliable detection of low-abundance cfRNAs without introducing contaminants that could inhibit downstream enzymatic reactions.

Notably, the Apostle MiniMax® Kit exhibited exceptional robustness with specialized cfRNA blood collection tubes, achieving up to 480-fold higher recovery for small RNAs in Streck cfRNA BCTs.

These findings underscore the kit's suitability for clinical research workflows, where sensitivity, reproducibility and compatibility with various sample types are critical for accurate cfRNA analysis and biomarker discovery.

## References

1. Apostle MiniMax® High Efficiency Cell-Free RNA Isolation Kit (200 µL x 50 preps per kit) D30451
2. Apostle MiniMax® High Efficiency Cell-Free RNA Isolation Kit (1 mL x 50 preps) D30452
3. Apostle MiniMax® High Efficiency cfDNA Isolation Kit (Standard Edition)



The Apostle MiniMax® cfRNA kit is for Research Use Only.

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