

# Apostle MiniMax™ cfDNA Isolation Kit

## User Validation Guide

Product	Cat #
Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard Edition)	A17622
Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Type S)	A17830

### Introduction

The Apostle MiniMax™ cfDNA Isolation Kit User Validation Guide serves as a reference for users to independently validate the performance of Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard Edition and Type S edition).

Specifically, this User Validation Guide provides experimental guidance on how to:

1. Validate the >95% recovery rate of DNA in plasma/serum, in terms of quantity.
2. Validate the >95% recovery rate of DNA in plasma/serum in downstream qPCR.
3. Quantify cfDNA yield and compare Apostle MiniMax™ cfDNA Isolation Kit with other technologies.
4. Troubleshooting for unexpected low yields, or genomic DNA contamination.

### 1. Validation of DNA recovery rate, in terms of quantity.

To validate isolation yield of DNA with sizes spanning 50bp - 3000bp, we suggest use the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit to isolate DNA standard ladder spiked in buffer/plasma/serum, and compare with the original DNA standard ladder before spike-in.

According to the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual, the sample lysis step (which involves Proteinase K treatment) can be skipped in many cases. However, for the DNA ladder isolation experiment, we recommend users perform the sample lysis step before spiking DNA ladder into plasma/serum. This is because the exogenous DNA ladder is subject to degradation in plasma/serum, which will lead to low DNA ladder isolation yield.

Below is an example procedure on validating DNA isolation yield in serum:

(1) Perform the sample lysis on 4 mL human plasma or fetal bovine serum (FBS, ThermoFisher, Cat# 26140079) according to the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual.

(2) During sample lysis, add 20 ul DNA ladder (50bp-3000bp, Sigma Aldrich Cat# S7025) to 80 ul TE buffer and mixed well by vortexing.

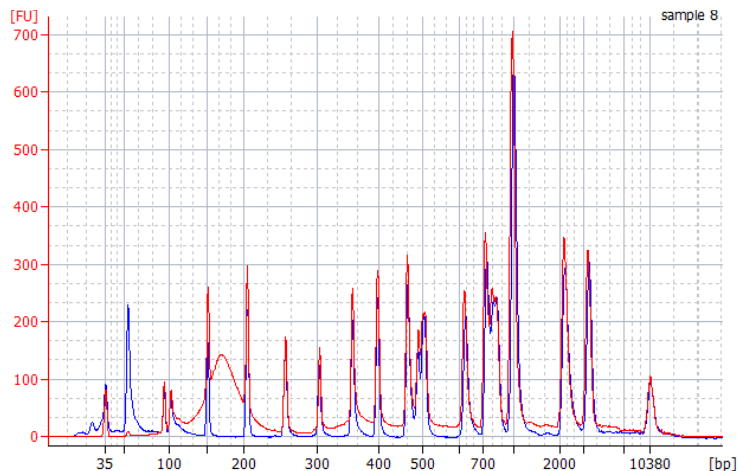
(3) After the sample lysis step, spike 50 ul of diluted DNA ladder into the plasma/serum; save the rest 50 ul of diluted DNA for recovery rate measurement.

(4) Perform DNA isolation according to the rest part of the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual, with a final elution volume of 50ul.

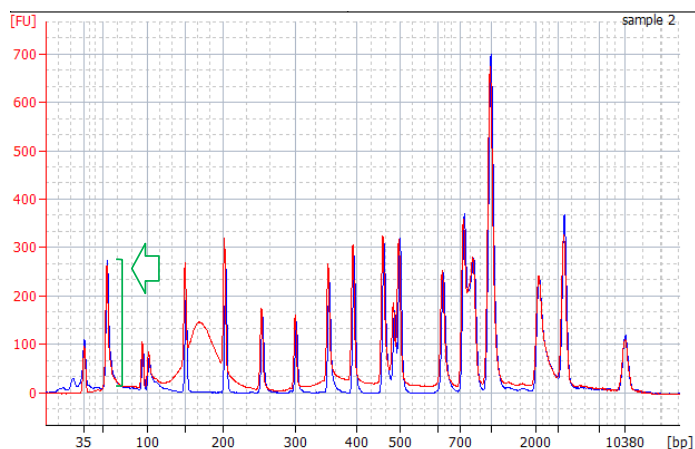
(5) Characterize and compare the isolated DNA and original diluted DNA with Bioanalyzer 2100 + High Sensitivity DNA Analysis Kit to calculate DNA recovery rate at different fragment sizes.

Since both spike-in and elution volume is 50 ul, a comparison of DNA concentration measured by Bioanalyzer 2100 means a direct comparison of DNA quantity, in order to calculate the DNA yield. It is of note that signals of the two samples must be normalized by the standard marker signal of the High Sensitivity DNA Analysis Kit, in order to compare with each other.

Exhibit 1-2 shows the typical DNA yield validation result for Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard edition and Type S edition):



**Exhibit 1. Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard edition): Over 95% DNA recovery in range between 80 – 3000bp.** DNA ladder was spiked in serum, followed by isolation with Apostle MiniMax™ High Efficiency cfDNA Isolation Kit. The isolated DNA was characterized by Bioanalyzer 2100 (red curve), and compared with original DNA ladder (blue curve). Apostle MiniMax™ High Efficiency cfDNA Isolation Kit offers excellent DNA recovery efficiency of > 95%.



**Exhibit 2. Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Type S edition): Over 95% DNA recovery in range between 50 – 3000bp.** DNA ladder was spiked in serum, followed by isolation with Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Type S). The isolated DNA was characterized by Bioanalyzer 2100 (red curve), and compared with the original DNA ladder (blue curve). Apostle MiniMax™ High Efficiency cfDNA Isolation Kit offers excellent DNA recovery efficiency of >95%, including small DNA fragments at ~50bp as highlighted.

## 2. Validation of DNA recovery rate, in downstream qPCR applications.

To quantitatively validate isolation yield of cfDNA in downstream applications like qPCR, we suggest use the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit to isolate synthetic DNA fragment (~170 bp) spiked in buffer/plasma/serum, and calculate the absolute quantity and recovery rate using standard curve protocol in qPCR study.

For synthetic DNA fragment isolation experiment, we recommend perform the sample lysis step

before spiking DNA fragment in plasma/serum, preventing exogenous DNA degradation from endogenous Dnase.

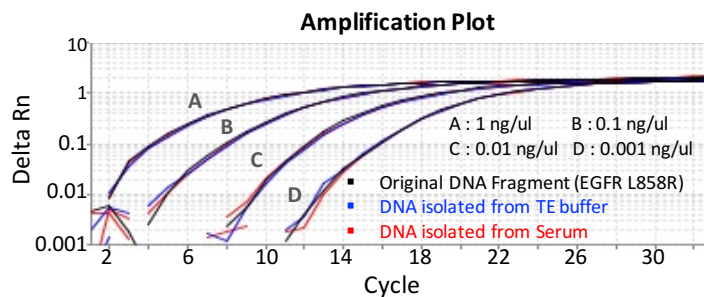
Below is an example experiment procedure using serum:

- (1) Use a synthetic double-strand DNA fragment containing the EGFR c.2573T>G L858R mutation (170bp, G-blocks® Gene fragment, IDT) to make a DNA standards dilution series at 1, 0.1, 0.01, 0.001 ng/ul.
- (2) Perform the sample lysis on 1mL human plasma or fetal bovine serum (FBS, ThermoFisher, Cat# 26140079) according to Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual. Repeat this step for 4 times to generate 5 aliquots of 1mL serum.
- (3) Spike 20 ul of each of the DNA fragment dilution series into each of the 1mL lysed plasma/serum, resulting in isolation working concentrations of 20 pg/ul, 2 pg/ul, 0.2 pg/ul, 0.02 pg/ul, respectively; save 20 ul of each of the DNA dilution series before spike-in for generation of standard curve in qPCR experiment.
- (4) Perform DNA isolation according to the rest part of Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual, with a final elution volume of 20 ul.
- (5) To quantify the isolated DNA fragments, perform qPCR on Applied Biosystems 7500 Fast Real-Time PCR System using the standard curve protocol.

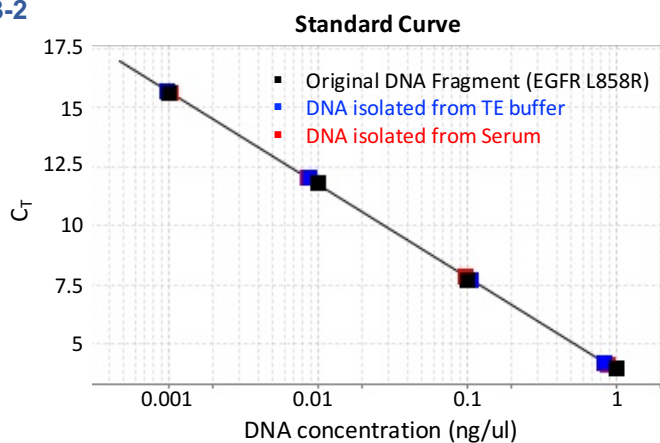
In brief, prepare a set of primers designed for this DNA fragment and SYBR green master mix according to manufacture's instruction, with the same volume (1 ul) of isolated DNA or original DNA solution series as the templates. The standard curve is automatically generated by the 7500 protocol using the  $C_T$  values of the original DNA fragment series. After that, determine the absolute quantity of isolated DNA by the standard curve, and calculate the recovery rate accordingly. It should be noted that PCR efficiency should be between 90-100%, indicated by a slope between -3.6 to -3.3 when performing a 10-fold dilution series experiment. Any unexpected low efficiency may indicate the existing of PCR inhibitors, or non-optimal experimental parameters.

Exhibit 3 shows the typical qPCR amplification plot and standard curve for experiment described above, using Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard edition):

### 3-1



### 3-2



**Exhibit 3. High performance of DNA mutation detection isolated with Apostle MiniMax™ High Efficiency cfDNA Isolation Kit.** DNA fragments dilution series were spiked in, isolated and used as templates in qPCR standard curve protocol as described above. **3-1** Amplification plot showing highly overlapping curves for mutated DNA fragment isolated with Apostle MiniMax™ High Efficiency Cell-Free DNA Isolation Kit and original DNA solution at different concentrations. **3-2** qPCR standard curve generated using original mutated DNA solution, in order to quantify the recovery of DNA isolated with Apostle MiniMax™ High Efficiency Cell-Free DNA Isolation Kit. DNA isolation recovery rate was calculated to be >90%, and the PCR efficiency is 96.8%.

Displayed DNA concentration series at 1, 0.1, 0.01, 0.001 ng/ul are the concentrations of the original DNA dilution series before spiking into 1mL of serum. The corresponding DNA isolation working concentrations are 20 pg/ul, 2 pg/ul, 0.2 pg/ul, 0.02 pg/ul, respectively.

### 3. Quantification of cfDNA yield and comparison of Apostle MiniMax™ cfDNA Isolation Kit with other technologies.

Various types of experiment can be performed to compare DNA yield and quality between Apostle MiniMax™ High Efficiency cfDNA Isolation Kit and other products. These experiments may include DNA ladder recovery, plasma/serum/urine cfDNA isolation, qPCR, sequencing, etc.

Since different products may use different protocols, we suggest users compare different technologies using the same sample, identical input volume, and identical elution volume, in order to get an accurate comparison result.

Particularly, we would recommend:

- (1) When characterizing isolated DNA using various types of methods (i.e. Bioanalyzer, qPCR etc.), we suggest users load identical volume of isolated DNA to make measurement.
- (2) It is not recommended to use spectroscopy methods (i.e. Qubit, Nanodrop) to quantify and compare cfDNA yields. Nanodrop has limited sensitivity for cfDNA characterization, and the reading of Qubit can be significantly affected by elution solution condition. Moreover, **carrier RNA introduced by some products significantly interfere with cfDNA quantification.** Carrier RNA can dominate the readings by Qubit/Nanodrop, and leads to falsely high cfDNA readings.
- (3) We also suggest users check the limit of detection (LOD) of the quantification method they may choose. For example, we recommend Bioanalyzer 2100 + High Sensitivity DNA Analysis Kit to characterize isolated cfDNA, as it has a LOD of 5 pg/ul.
- (4) It is also necessary to normalize signal by internal control (such as the marker signal of Bioanalyzer), in order to accurately compare DNA quantity from different samples.

## 4. Troubleshooting Guides

Issues	Possible reason	Suggested solution
Low yield	Insufficient amount of working-condition magnetic nanoparticles	Store magnetic particles properly at 2-8 °C without freezing. Allow particles reach room temperature before use. Vortex the nanoparticles to fully resuspend particles before use. May need to sonicate the nanoparticle solution to resuspend the particle if the particle was frozen due to shipping or other improper storage.
	Improper storage of the kit	Store magnetic particles at 2-8 °C, store other components at room temperature, and protect from light. All other solutions stored at room temperature (15-30°C) should be clear solution. If precipitate is observed in any reagent, warm the solution to 37°C until the precipitate dissolves.
	cfDNA degradation during sample lysis step	Order high quality Proteinase K for sample lysis to avoid cfDNA degradation. Or skip the sample lysis step when possible.
	Loss of magnetic nanoparticles during process	Minimize nanoparticle loss during process. Make sure all particles are transferred properly during the isolation process. Make sure no particles left in the supernatant when removing supernatant.
	Undried magnetic nanoparticles after 2 <sup>nd</sup> wash bringing ethanol to the elution step	Make sure the residual 2 <sup>nd</sup> wash solution is evaporated after the 2 <sup>nd</sup> wash step. However, avoid over-drying nanoparticles, as it will affect the resuspension of the nanoparticles.
	Wash buffer was not prepared according to the manual	Prepare wash buffers properly, by adding 100% Ethanol or Isopropanol, according to the manual.
Genomic DNA contamination	Genomic DNA releasing to plasma/serum/urine/ saliva during sample preparation step	Prepare plasma/serum/urine/saliva samples properly, by completely removing cells or cell debris. Avoid cell breakage.