EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit

Base Catalog # P-1065

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit utilizes magnetic beads-based size-fractionation technology to isolate circulating cell-free DNA (ccfDNA) from plasma/serum samples in a simple and fast manner. The isolated ccfDNA can be directly used for real time-PCR and DNA library preparation suitable for next generation sequencing.

Starting Material and Input Amount: Plasma or serum from various species. Input amount can be from 0.1 – 1 ml; however, the standard input amount is 0.5 ml per sample. The ccfDNA yield is dependent on the amount contained in the plasma or serum. In general, >80% of total ccfDNA contained in plasma/serum can be obtained using this kit.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	ID	Cat. #P-1065-25 (25 Isolations)	Cat. #P-1065-50 (50 Isolations)	Storage Upon Receipt
Proteinase K	PK	0.7 ml	1.4 ml	4°C
cfDNA Capture Enhancer	cCE	0.4 ml	0.8 ml	4°C
cfDNA Capture Beads	cCB	60 µl	120 µl	4°C
Capture Buffer	CB	14 ml	28 ml	4°C
Elution Buffer	EB	0.6 ml	1.2 ml	4°C

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C. Each component of the kit is sufficient for the indicated isolation quantity using the standard input amount (0.5 ml per sample).

Upon receipt: Store the following components at 4°C: **cfDNA Capture Beads**, **cfDNA Capture Enhancer**, **Capture Buffer**, **Proteinase K** and **Elution Buffer**. Store all other components at room temperature. The kit is stable for at least 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Agilent® Bioanalyzer® or comparable method to assess the size of DNA
- ☐ Thermocycler with 48 or 96 well block
- ☐ Magnetic stands (suitable for 1.7 ml microtube, 0.2 ml PCR tube and 96-well plate)
- ☐ Pipettes and pipette tips
- ☐ 1.7 ml microcentrifuge tube
- ☐ 96-well microplate
- ☐ 90% Ethanol
- ☐ 80% Ethanol
- ☐ Plasma or serum sample

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

Genetic and epigenetic analysis of circulating cell-free DNA (ccfDNA) in plasma/serum or other body fluids provides unique opportunities for early detection of a wide range of clinical disorders such as cancer, autoimmune disease, infection and fetal disorders. It was demonstrated that ccfDNA of clinical importance occurs predominantly as fragments of approximately 170 bases from mononucleosomes with a smaller proportion as fragments of 360 bases from di-nucleosomes [1,2]. Such nucleosomal complexes are released into blood circulation during apoptotic cell death and will be increased under various pathological circumstances such as inflammation, pulmonary embolism, autoimmune disease, and cancer [3,4]. It is also shown that ccfDNA from nucleosomal complexes in serum and plasma is small size fragment DNA (170-500 bps) and using such ccfDNA for genetic or epigenetic analysis provides better and more accurate identification of physiological and pathological status [5].

There are several methods currently being used for ccfDNA isolation from plasma and serum. These methods are based on the capture of DNA by silicone column binding, phenol-chloroform separation or silica beads, and are time consuming and low throughput. To address these problems, EpigenTek offers the EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit for ccfDNA isolation. The kit has the following features:

- Fast and straightforward procedure can be finished within 1 hour.
- Uses innovative magnetic bead-based size-fractionation technology for isolation of ccfDNA from plasma/serum in a simple and convenient manner.
- The isolated DNA can be directly used for both qPCR and NGS DNA library preparation.
- Efficient removal of proteins, salts, nucleases, PCR inhibiting substances, and other impurities such as polysaccharides, polyphenols and lipids.

- Sensitive and efficient DNA capture enables successful isolation with high recovery (>80% of input mononucleosomal DNA), even when the quantities of starting material are limited (as low as 0.2 ml).

References

1. Jahr S et al: Cancer Res. 2001, 61: 1659-1665
2. Suzuki N et al: Clin Chim Acta. 2008, 387: 55-58
3. Holdenrieder S et al: Crit Rev Clin Lab Sci. 2009, 46: 1-24
4. Schwarzenbach H et al: Nat Rev Cancer. 2011, 11: 426-437
5. Chan KCA et al: Clinical Chem. 2004, 50: 88-92

PRINCIPLE & PROCEDURE

The EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit contains all components which have been optimized for the simple and rapid isolation of small size cfNA from plasma/serum. The circulating nucleosomal complexes are first digested and DNA is then enzymatically released. The ccfDNA is efficiently captured via size-fractionation magnetic beads (ccfDNA Capture Beads) by applying the beads to a magnetic field (EpiMag™ HT (96-Well) Magnetic Separator, Cat. #Q10002-1, or similar). The captured ccfDNA is purified by simply washing the beads. The purified ccfDNA is then eluted from the beads for immediate use or storage.

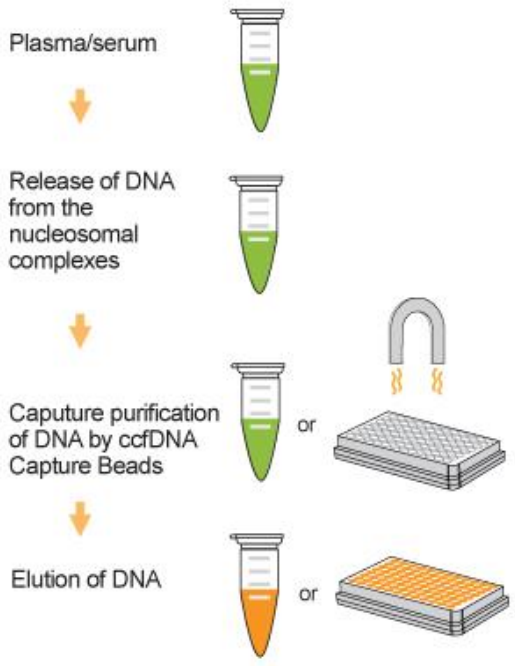

Fig 1. Workflow of EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit.

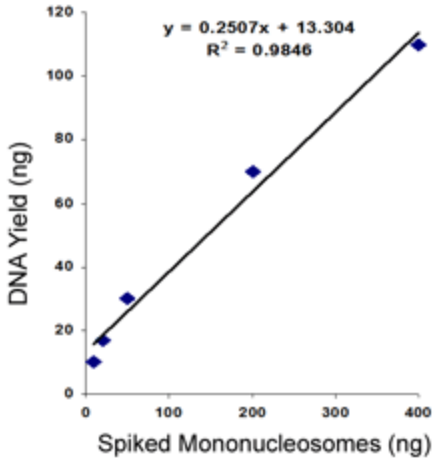

Fig 2. High recovery of ccfDNA: Different amounts of HeLa mononucleosomes were spiked into 0.5 ml of plasma then isolated using the EpiQuik™ Circulating Cell-Free DNA (ccfDNA) Isolation Easy Kit. The isolated DNA was fluorescently quantified.

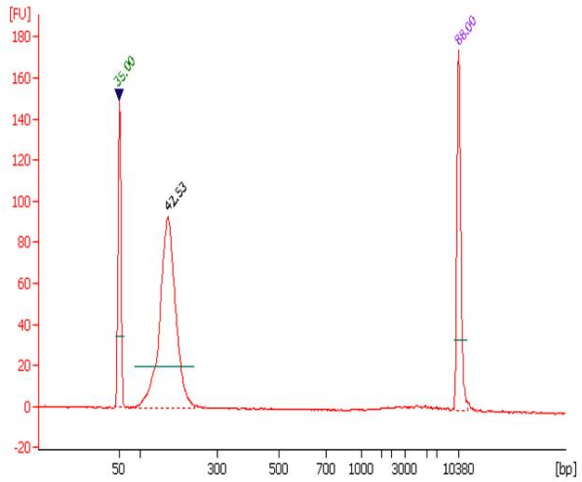

Fig 3. High recovery of ccfDNA confirmed by bioanalyzer analysis: 500 ng of Hela polynucleosome (around 3000 bps) and 500 ng of mononucleosome (around 170 bps) were simultaneously spiked into 0.5 ml plasma and then isolated and size-selected.

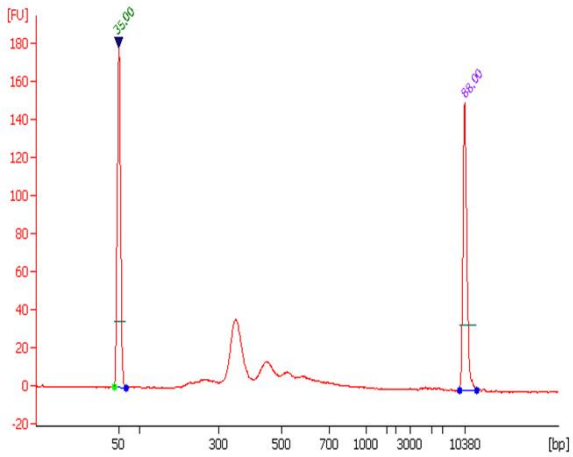

Fig 4. Bioanalyzer trace of DNA library prepared from 20 ng of purified DNA isolated from plasma spiked with both mono- and poly-nucleosomes. Library peak size: 350 bps.

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Both fresh and frozen plasma/serum from various sources can be used. However, fresh plasma/serum will generally give higher DNA yields than frozen. Furthermore, frozen plasma/serum will lead to DNA loss of about 10% per year. The input volume of plasma/serum can be from 0.1-1 ml with the standard volume of 0.5 ml per sample. If serum sample is used, the serum should be prepared within 6 hours after blood draw, since lysis of peripheral blood lymphocytes may cause an artificial increase in the amount of DNA during serum separation.

For the magnetic stand used to capture DNA bound to the magnetic beads, we recommend using EpigenTek's EpiMag™ HT (96-Well) Magnetic Separator (Cat. #Q10002-1), which has very strong magnetic intensity to quickly and efficiently achieve high, reproducible retention of magnetic bead-bound DNA in various 96-well plates. The separator can also be used with 1.7 ml microcentrifuge tubes with volumes greater than 300 µl.

1. ccfDNA Capture

a. Add maximum 0.5 ml of plasma/serum into each 1.7 ml microcentrifuge tube followed by adding 15 µl of **cfDNA Capture Enhancer**, 20 µl of **proteinase K.** Mix and incubate at 60˚C for 15 min. Meanwhile, prepare **cfDNA Binding Solution** based on the sample number needed: Add 2 µl of **cfDNA Capture Beads** per 500 µl of **Capture Buffer** and then mix well by pipetting up and down 10-20 times.

b. Add 550 µl of **cfDNA Binding Solution** into each sample tube. Mix by pipetting up and down at least 10 times and incubate at room temperature for 10 min with rotation at 10-15 rpm. If more than 1 ml of plasma/serum from the same sample is used, use additional tube with a maximum of 0.5 ml plasma/serum per tube.

c. Place the tube on the EpiMag™ HT (96-Well) Magnetic Separator (EpigenTek Cat. #Q10002-1) or an appropriate magnetic separation stand for 10 min or until the solution is clear. Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA)*

2. ccfDNA Purification

a. Add 500 µl of *90% ethanol* solution to the tube. Place the tube on the magnetic stand for 1 minute or until the solution is clear. Remove and discard supernatant.

b. Repeat Step a one time for a total of two washes. Make sure that the ethanol is completely removed after the last wash.

c. Air dry beads at room temperature for 1-2 minutes while the tube is on the magnetic stand. It is important to ensure all traces of ethanol are removed.

Note: *Take care not to over dry the bead spot (an over dried bead spot appears cracked) as this will significantly decrease elution efficiency.*

d. Resuspend the beads in 20 µl **Elution Buffer**, and transfer the solution containing the beads into the wells of a 96-well microplate. Incubate at room temperature for 6 minutes to release the DNA from the beads.

e. Capture the beads by placing the plate on the magnetic stand for 2 minutes or until the solution is completely clear.

Note: *It is normal to see that the eluted solution may be slightly yellow or cloudy.*

f. Transfer the supernatant to a new 0.2 ml PCR tube or PCR plate and measure the amount of DNA using a fluorescent method (ex: use EpigenTek's FitAmp™ General DNA Quantification Kit, Cat. #P-1020, or Picogreen assay). If necessary, the fragment size of the isolated DNA can be measured using an Agilent® Bioanalyzer® or comparable method.

g. The purified ccfDNA can now be used for a downstream application or stored at -20ºC for later use.

APPENDIX

In general, isolated ccfDNA is small fragment DNA. Occasionally, a significant amount of large fragment DNA (>1,000 bps) exists, indicating possible contamination caused when genomic DNA is released from lysed nucleated blood cells during blood collection or plasma/serum preparation. Under this condition, the small fragment ccfDNA can be size-selected using **MQ Binding Beads** from the EpiNext™ DNA Purification HT System (EpigenTek Cat. #P-1063, sold separately) according to the following steps:

a. Resuspend **MQ Binding Beads** by vortex.

b. Add 0.5X (0.5:1) resuspended **MQ Binding Beads** to the isolated DNA sample solution (ex: 10 µl of MQ beads to 20 µl of sample). Mix well by pipetting up and down at least 10 times.
c. Incubate for 6 minutes at room temperature.
d. Put the tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). If the magnetic stand is not suitable for the PCR tube, transfer the beads solution to an appropriate tube or plate well that is compatible with the magnetic stand. Carefully transfer the supernatant containing DNA to a new tube *(Caution: do not discard the supernatant, which contains the desired DNA fragment size).* Discard the beads that contain the unwanted large fragments.
e. Add 1X resuspended **MQ Binding Beads** to the supernatant (ex: 30 µl of MQ beads to 30 µl of supernatant). Mix well and incubate for 5 minutes at room temperature.
f. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*
g. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 minute and then carefully remove and discard the ethanol.
h. Repeat Step g one time, for a total of two washes.
i. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
j. Resuspend the beads in 10-20 µl **Elution Buffer** and incubate at room temperature for 2 minutes to release the DNA from the beads.
k. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
l. Transfer 10-20 µl of eluted DNA to a new 0.2 ml PCR tube for PCR amplification.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of isolated DNA	Insufficient amount of starting material.	Increase the volume of plasma/serum for ccfDNA isolation.
	Low concentration of ccfDNA in the samples.	Sample was left at room temperature for a long time or the sample itself contains a low amount of ccfDNA. Increase the volume of the sample for re-isolation.
	Improper storage of the kit.	Ensure that the kit has not exceeded the expiration date. The standard shelf life, when stored properly, is 6 months from date of receipt.
	cfDNA Capture Beads are not well suspended at Step a of ccfDNA capture.	Completely suspend the beads to allow maximal DNA release.
	DNA degradation due to improper anticoagulant in blood tube.	Use new blood sample in EDTA blood tube for plasma/serum separation.
	Sample has been subjected to too many freeze/thaw cycles.	Repeated sample freezing and thawing may lead to DNA degradation. Always use fresh samples or samples thawed only once.

	Low-percentage ethanol used at DNA purification steps.	Freshly prepared *90% ethanol* should be used.
Presence of larger fragments (>1,000 bps) than expected.	Lysis of peripheral blood lymphocytes during plasma/serum separation.	The serum should be prepared as soon as possible after blood draw and the separation time short -- no more than 6 hours.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003 FitAmp™ General Tissue Section DNA Isolation Kit
P-1006 DNA Concentrator Kit
P-1009 FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017 FitAmp™ Urine DNA Isolation Kit
P-1018 FitAmp™ Blood and Cultured Cell DNA Extraction Kit
P-1020 FitAmp™ General DNA Quantification Kit

PCR Analysis

P-1028 Methylamp™ MS-qPCR Fast Kit
P-1029 EpiQuik™ Quantitative PCR Fast Kit

DNA Library Prep

P-1051 EpiNext™ DNA Library Preparation Kit (Illumina)
P-1053 EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina)
P-1055 EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina)
P-1056A EpiNext™ High-Sensitivity Bisulfite-Seq Kit (Illumina)
P-1059 EpiNext™ DNA Size Selection Kit
P-1063 EpiNext™ DNA Purification HT System

Magnetic Devices

Q10002 EpiMag™ HT (96-Well) Magnetic Separator