EpiQuik™ Circulating Cell-Free DNA Isolation Kit

Base Catalog # P-1064

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Circulating Cell-Free DNA Isolation Kit utilizes magnetic beads based size-fractionation technology to isolate circulating cell-free DNA (ccfDNA) from mono-and di-nucleosomal complexes in plasma/serum samples. 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	Cat. #P-1064-25 (25 Isolations)	Cat. #P-1064-50 (50 Isolations)	Storage Upon Receipt
ccfDNA Capture Beads	4 ml	8 ml	4°C
ccfDNA Capture Enhancer*	0.7 ml	1.4 ml	4°C
Capture Buffer	23 ml	23 ml X 2	4°C
Digestion Solution	2 ml	4 ml	4°C
Proteinase K*	60 µl	120 µl	4°C
MQ Binding Beads	3 ml	6 ml	4°C
Elution Buffer	0.6 ml	1.2 ml	4°C
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** Spin the solution down to the bottom prior to use.*

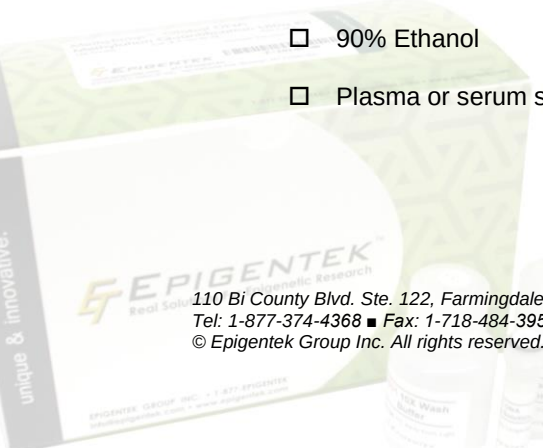
SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part 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: **ccfDNA Capture Beads**, **ccfDNA Capture Enhancer**, **Capture Buffer**, **Digestion Solution**, **Proteinase K**, **MQ Binding Beads** and **Elution Buffer**. Store all other components at room temperature.

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
- ☐ 0.2 ml or 0.5 ml PCR tube
- ☐ 96-well microplate (optional)
- ☐ 90% Ethanol
- ☐ Plasma or serum sample



GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiQuik™ Circulating Cell-Free DNA Isolation 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. Thus, only use the User Guide that was supplied with the kit when using that kit.

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

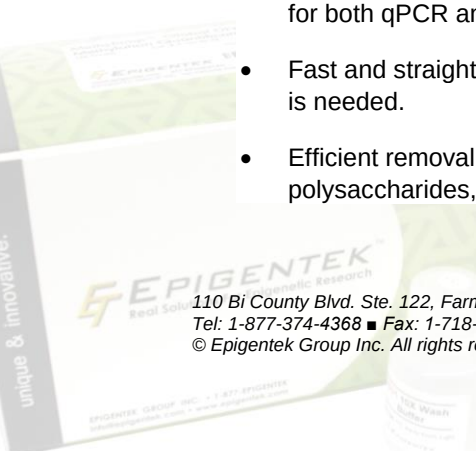
Intellectual Property: The EpiQuik™ Circulating Cell-Free DNA Isolation Kit and methods of use contain proprietary technologies by Epigentek.

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 using ccfDNA from such nucleosomal complexes 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. All of these methods are based on capture of DNA by silicone column binding or phenol-chloroform separation. The DNA isolated by these methods contains both ccfDNA and non-ccfDNA, which may affect the accuracy of downstream analysis. To address these problems, Epigentek offers the EpiQuik™ Circulating Cell-Free DNA Isolation Kit for ccfDNA isolation. The kit has the following features:

- Uses innovative magnetic bead based size-fractionation technology for selective isolation of circulating cell-free DNA from plasma/serum that is mainly 170 bps in size. The isolated DNA can be directly used for both qPCR and NGS DNA library preparation.
- Fast and straightforward procedure can be finished within 2 hours. No gels, columns or centrifugation is needed.
- 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 monoucleosomal DNA), even when the quantities of starting material are limited (as low as 0.1 ml).
- Manual and automation friendly – Scalable for single tube or 96-well plate formats.

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 Isolation Kit contains all components which have been optimized for the simple and rapid isolation of small size nucleosomal DNA from plasma/serum. The mono- and di-nucleosomal complexes are 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 nucleosomal DNA is then enzymatically released, and purified using MQ Binding Beads by simply washing the beads. The purified ccfDNA is then eluted from the beads for immediate use or storage.

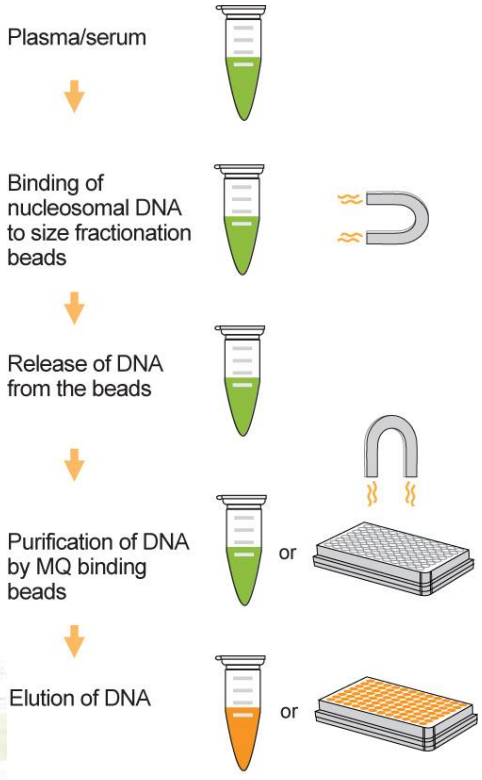


Fig 1. Workflow of the EpiQuik™ Circulating Cell-Free DNA Isolation Kit.

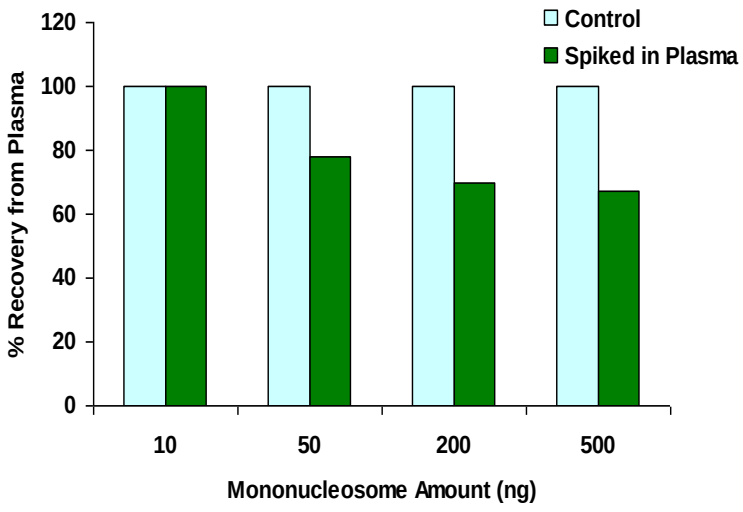


Fig 2. High recovery of ccfDNA: Different amounts of HeLa mononucleosomes were spiked into 0.5 ml of plasma then isolated using the EpiQuik™ Circulating Cell-Free DNA Isolation Kit. The isolated DNA was fluorescently quantified using control DNA which was directly isolated from the same amount of mononucleosomes.

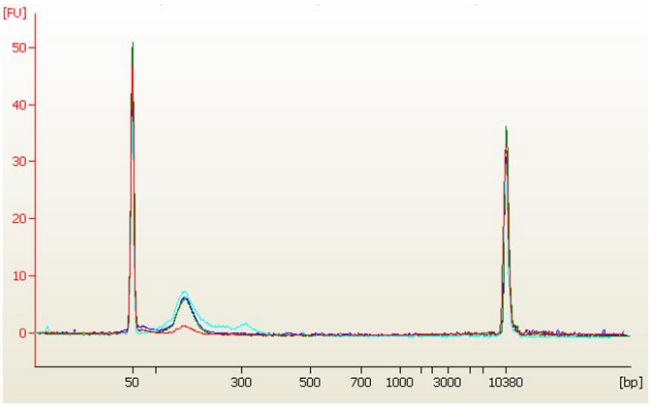


Fig 3. High recovery of ccfDNA confirmed by bioanalyzer analysis: Different amounts of HeLa mononucleosome were spiked into 0.5 ml of plasma then isolated: red: 200 ng; deep blue and green: 500 ng; sky blue: 500 ng of unpurified HeLa mononucleosome as the control. Isolated DNA fragment size: 170 bps.

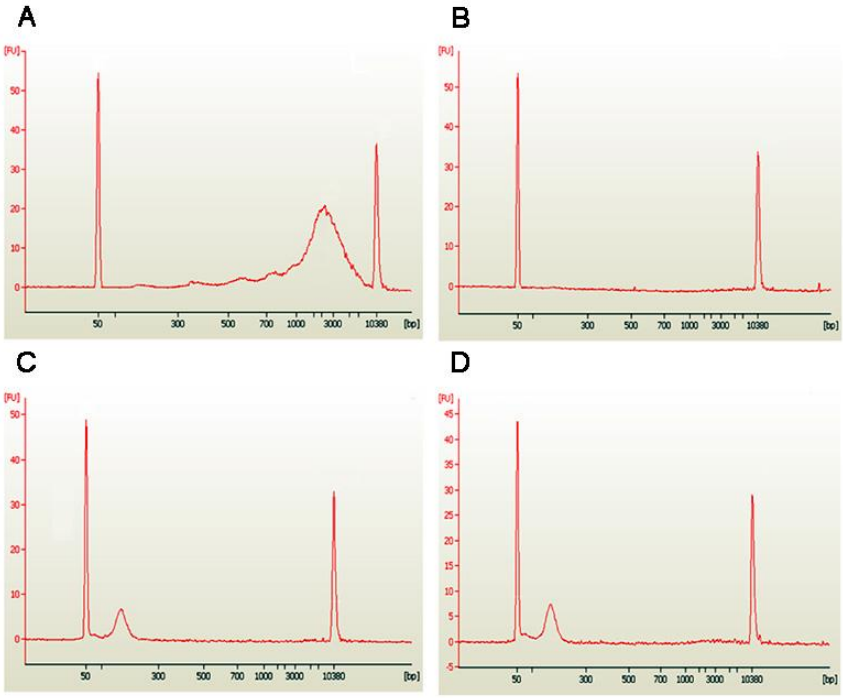


Fig 4. High selectivity of specifically isolating small size ccfDNA (mononucleosomal DNA): Panel A: 500 ng of unspiked control polynucleosome (up to 2000 bps); Panel B: 500 ng of the same polynucleosome spiked in 0.5 ml plasma; Panel C: 500 ng of mononucleosome spiked in 0.5 ml plasma; Panel D: 500 ng of polynucleosome and 500 ng of mononucleosome, which were simultaneously spiked into 0.5 ml plasma.

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 for capturing DNA bound to magnetic beads, we recommend using Epigentek's EpiMag™ HT (96-Well) Magnetic Separator (Cat. #Q10002-1), which has very strong magnetic intensity for

quickly and efficiently achieving 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 24 µl of **ccfDNA Capture Enhancer**, 900 µl of **Capture Buffer** and 50 µl of **ccfDNA Capture Beads** (make sure the beads have been thoroughly resuspended before use). Mix by pipetting up and down at least 20 times and incubate at room temperature for 10 min. If more than 1 ml of plasma/serum from the same sample is used, use additional tubes with a maximum of 0.5 ml plasma/serum per tube.

b. Place the tube in a bench-top centrifuge (ex: Eppendorf 5415D) and centrifuge at 12000 rpm for 5 min to spin down the beads to the side of the tube's bottom. Place the tip of the tube into the wells of the EpiMag™ HT (96-Well) Magnetic Separator (Epigentek Cat. #Q10002-1) until it is firmly in place, or with an appropriate magnetic separation stand, to carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA)*

c. While the tube is on the magnetic separator, prepare **DNA Release Solution** by adding 2 µl of **Proteinase K** to each 40 µl of **Digestion Solution**.

d. Keep the tube in the magnetic stand and add 40 µl of **DNA Release Solution** to each tube. Resuspend the beads. (This can be done by repeatedly pipetting the 40 µl of **DNA Release Solution** up and down onto the beads attached to the wall of the tube). Transfer the beads solution to a 0.2 ml or 0.5 ml PCR tube. Incubate at 55˚C for 10 minutes to release the DNA from the beads.

e. Capture the beads by placing the tube into the EpiMag™ HT (96-Well) Magnetic Separator or an appropriate magnetic stand until the solution is clear (about 5 minutes; if the magnetic stand is not suitable for the tube, transfer the beads solution to an appropriate tube or plate well that is compatible to your magnetic stand). Carefully transfer the supernatant that contains DNA to a new 0.2 ml or 0.5 ml PCR tube or a U-bottom 96-well microplate. *(Caution: **DO NOT** discard the supernatant. Discard the beads.)*

2. ccfDNA Purification

a. Resuspend **MQ Binding Beads** by vortexing or shaking. Add 2X (2:1 ratio) resuspended beads to the DNA sample (ex: 80 µl of MQ beads to 40 µl of DNA solution). Mix thoroughly by pipetting up and down at least 10 times.

b. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.

c. Put the tube/plate on the EpiMag™ HT (96-Well) Magnetic Separator or an appropriate magnetic stand until the solution is clear (about 5 minutes; if the magnetic stand is not suitable for the tube/plate, transfer the beads solution to an appropriate tube or plate well that is compatible to your magnetic stand). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA)*

Note: *If EpiMag™ HT (96-Well) Magnetic Separator is used for 0.2 or 0.5 ml tubes, the adaptor should be used. See the instructions for use of the EpiMag™ HT (96-Well) Magnetic Separator (Cat. #Q10002-1).*

d. Add 200 µl of *90% ethanol* solution to each tube/well and resuspend the beads. Place the tube/plate on the magnetic stand for 1 minute or until the solution is clear. Remove and discard supernatant.

e. Add 200 µl of *90% ethanol* solution to each tube/well. Place the tube/plate on the magnetic stand for 1 minute or until the solution is clear. Remove and discard supernatant. Make sure that the ethanol is completely removed after the last wash.

f. Air dry beads at room temperature for 2-3 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.*

g. Resuspend the beads in 20 µl **Elution Buffer**, and incubate at room temperature for 4 minutes to release the DNA from the beads.

h. Capture the beads by placing the tube/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.*

i. 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.

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

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.

	ccfDNA Capture Beads are not well suspended at step d of ccfDNA capture.	Completely suspend the beads to allow maximal DNA release.
	Improper ratio of MQ beads to DNA volume during purification.	Check if the correct volume of **MQ Binding Beads** was added to the DNA solution at Step 2a. Proper ratios should capture fragments >100 bps.
	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 (>5,000 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 h.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1007	FitAmp™ Gel DNA Isolation 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