EpiMag™ High Throughput DNA Isolation Universal Kit

Base Catalog # P-1022

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiMag™ High Throughput DNA Isolation Universal Kit utilizes magnetic bead technology for high throughput DNA isolation from various biological samples, which include tissues, cultured cells, blood, bone marrow, mouth wash, buffy coat, urine, cerebrospinal fluid, buccal scrapes, sputum, bacteria, fungi, plants, and feces.

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	96 Samples Cat. #P-1022-96	Storage Upon Receipt
LB (Lysis Buffer)	25 ml	RT
PK (Proteinase K)	1 Vial	4°C
DB (Digestion Buffer)	1.1 ml	RT
DNB (DNA Binding Buffer)	6 ml	RT
Magbeads	1 ml	4°C
EB (Elution Buffer)	2 ml	RT

SHIPPING & STORAGE

The kit is shipped at ambient room temperature.

Upon receipt: Store the following components at 4°C: **Proteinase K,** and **Magbeads.** Store all other components at room temperature.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Magnetic stand (96-well format)
- ☐ Pipettes and pipette tips
- ☐ 100% Isopropanol
- ☐ 90% Ethanol
- ☐ Rnase A
- ☐ Sample of interest

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiMag™ High Throughput DNA Isolation Universal 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 EpiMag™ High Throughput DNA Isolation Universal Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiMag™ High Throughput DNA Isolation Universal Kit and methods of use contain proprietary technologies by EpigenTek.

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A BRIEF OVERVIEW

A rapid and cost-efficient genomic DNA extraction for different materials is quite important for various downstream applications. There are many DNA extraction methods and protocols, most of which have been claimed to be reproducible with relatively good yields of high-quality DNA. Nonetheless, these methods can only be used for a specific starting material and are not fully applicable to various types of materials. Therefore, the costs can be relatively high, and they are inconvenient if DNA is required to be isolated from multiple different materials. In addition, these methods remain time-consuming and have low throughput. Thus, to address these impediments, EpigenTek developed the EpiMag™ High Throughput DNA Isolation Universal Kit to extract high-quality DNA from different materials. This kit has the following features:

- Optimized buffer chemistries for completely separating DNA from multiple materials, including various tissues, cultured cells, blood/bone marrows, buffy coat, urine, cerebrospinal fluid, buccal scrapes, feces, bacterial, fungi, and plants.
- Cost-effective, fast, and straightforward procedure can be finished within 45 minutes for 96 samples and is highly amenable to various automation platforms. No gels or columns are needed.
- Efficient removal of protein, salts, enzymes, 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 DNA), even when the quantities of starting material are limited.
- Manual and automation-friendly - scalable for single tube or 96-well plate formats.

PRINCIPLE & PROCEDURE

The EpiMag™ High Throughput DNA Isolation Universal Kit contains all the optimized components needed for the simple and rapid isolation of PCR-ready genomic DNA from small quantities of various crude sample materials. The specifically designed magnetic beads allow for efficient and quick binding of the DNA to the beads after cell lysis. The DNA bound to the beads is pulled to the side wall of the well by applying a magnetic field (EpiMag HT magnetic separator (Q10002-1) or similar). DNA is purified by simply washing to remove any contaminants and potential PCR inhibitors. Purified DNA is then eluted from beads for use or storage.

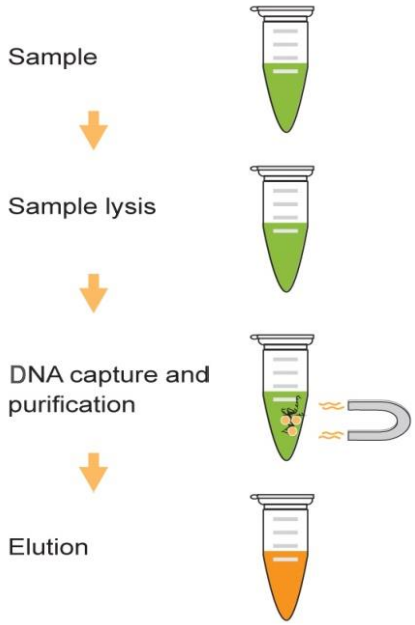

Fig 1. Workflow of the EpiMag™ High Throughput DNA Isolation Universal Kit

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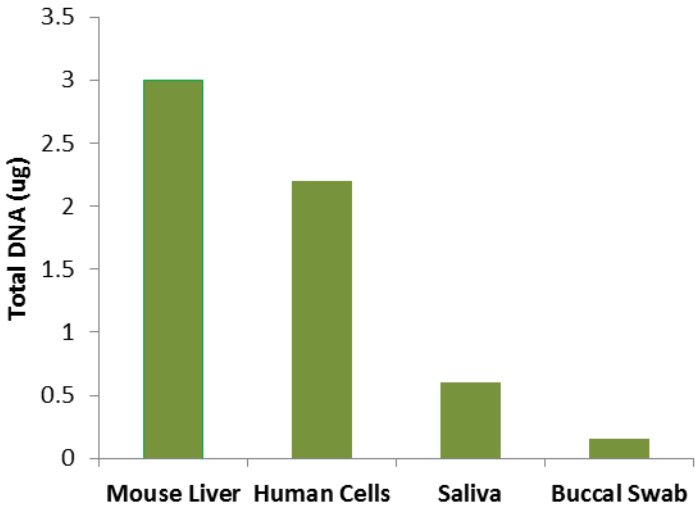

Fig 2. Isolated DNA from different samples. 5 mg mouse tissue, 10^6 human cells, 80 µl human saliva, and 1 buccal swab rotated in TE buffer were used for DNA isolation by using EpiMag™ High Throughput DNA Isolation Universal Kit

ASSAY PROTOCOL

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

1. Pre-treatment of the samples

Tissues (<10 mg)

Harvest 10 mg or less of various tissues into a 1.5 ml tube and add nitrogen liquid to immerse tissue. While the nitrogen liquid is still evaporating, very gently crush the tissue clumps into small pieces with a pestle for a 1.5 ml tube. When the nitrogen liquid has nearly evaporated, vigorously grind the tissue pieces into fine powder. The DNA yield is dependent on the tissue types; in general, 1-3 ug for 10 mg of lung and heart, 3-10 ug for 10 mg of spleen, brain, kidney, and liver.

Cultured Cells (<1 × 10^6)

Adhesive cultures: Cells (no more than 1 × 10^6) are detached by trypsinization and collected into a 1.5 ml vial. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 ml of PBS once by centrifugation at 2000 rpm for 3 minutes.

Suspension cells: Cells (no more than 1 × 10^6) are directly collected into a 1.5 ml vial. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 ml of PBS once by centrifugation at 2000 rpm for 3 minutes.

In general, 2-3 ug of DNA could be yielded from 1 × 10^6 cultured cells.

Blood and Bone Marrow (< 30 ul)

30 µl or less of fresh anticoagulated blood (EDTA/ACD/citrate-treated) or bone marrow can be used directly for DNA isolation. Around 1 ug of DNA could be yielded from 30 ul of blood and bone marrow.

Buffy Coat

20 µl or less of buffy coat can be used directly for DNA isolation. 1-2 ug of DNA could be yielded from 30 ul of buffy coat.

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Urine

Collect 5 ml of fresh urine into a 15 ml conical tube and centrifuge at 2000 rpm for 10 minutes to pellet cells. Remove supernatant and resuspend the pellet with 1 ml PBS. Transfer the cell suspension to a 1.5 ml tube and centrifuge at 3000 rpm for 2 min to pellet the cells. The DNA yield is dependent on the cell number contained in the urine. In general, the number of cells in the urine may have a range of 1000–10000 nucleated cells/ml.

Note: *If the urine sample is not processed within a day of sample collection, protease inhibitors must be added*.

Cerebrospinal Fluid

Collect 2 ml of fresh cerebrospinal fluid into a 15 ml conical tube and centrifuge at 2000 rpm for 10 minutes to pellet cells. Remove supernatant and resuspend the pellet with 1 ml PBS. Transfer the cell suspension to a 1.5 ml tube and centrifuge at 3000 rpm for 2 min to pellet the cells. The DNA yield is dependent on the cell number contained in the CSF. In general, the number of cells in the CSF may range from 5–3000 cells/ml, but it is usually 100–400 cells/ml with lymphocyte predominance.

Buccal Scrapes

Collect buccal scrapes using a simple plastic scraper. Place the scraper in a microcentrifuge tube with 50 µL PBS and spin down the sample. Remove the PBS and the scraper.

Saliva

Collect 80 ul of saliva and add into 0.2 ml PCR tubes. Typically, around 1 ug of DNA can be yielded from 80 ul of saliva.

Bacteria/Fungi

Collect 5 mg (wet weight) of bacterial or fungi into a 1.5 ml tube. It equates to approximately 1 x 10^8 bacterial cells or 1 x 10^7 fungi. Pellet bacteria/fungi by microcentrifugation at 15,000 × g for 5 min. Typically, up to 3 ug of DNA can be yielded from 1 × 10^8 bacterial cells or 1 x 10^7 fungi cells.

Plants

Approximately 30–100 mg fresh plant leaf is used per isolation. Homogenize the plant leaf for 2 min in liquid nitrogen with a pestle to mechanically break open the hard cell walls and thereby increase DNA yield.

DNA Isolation

a. Prepare **Proteinase K Solution** by adding 1 ml of **DB (Digestion Buffer)** to **PK (Proteinase K)** vial. Vortex until the solution is clear.

b. Prepare **DNA Isolation Solution** by adding 8 ul of **Proteinase K Solution** to each 72 µl **LB** (**Lysis Buffer)**. Add 80 µl of **DNA Isolation Buffer** into sample tubes to resuspend cell pellets. For saliva samples, add 40 µl of **DNA Isolation Buffer.** Vortex 10 seconds and incubate at 60°C for 20 minutes.

c. Transfer the solution into the wells of a 96-well microplate followed by adding 70 µl of **DNB (DNA Binding Buffer)** and 150 µl of isopropanol**.** Resuspend **Magbeads** by vortexing. Add 5 µl of the resuspended beads to each well, mix, and incubate at room temperature for 5 min.

d. Put the plate in the magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads containing DNA).*

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e. Keep the plate in the magnetic device, add 200 µl of freshly prepared 90% ethanol to the wells, then carefully remove and discard the supernatant.

f. Repeat Step e twice for a total of three washes.

g. Air dry beads for 2 minutes at Room Temperature while the plate is on the magnetic stand. It is to ensure all traces of Ethanol are removed.

***Note**: Make sure not to over dry the bead spot (Over dried bead spot appears cracked) as this will significantly decrease elution efficiency.*

h Resuspend the beads in 20 µl **EB (Elution Buffer)** and incubate at room temperature for 5 minutes to release the DNA from the beads.

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

j. Transfer 20 µl of supernatant to a new 0.2 ml PCR tube or plate for immediate use or storage at -20ºC after tightly capping the PCR tube or plate.

***Note:** If necessary, the RNase can be used to further purify DNA according to the manufacturer's instruction before use or storage.*

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of purified DNA	Insufficient amount of starting DNA.	To obtain the best results, the amount of input DNA should be >10 ng.
	Insufficient purity of starting DNA.	Remove RNA by RNAse treatment
	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.
	Too many cycles of sample freezing and thawing.	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	80% of freshly prepared ethanol should be used.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003 FitAmp™ General Tissue Section DNA Isolation Kit
P-1004 FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006 DNA Concentrator Kit
P-1009 FitAmp™ Paraffin Tissue Section DNA Isolation Kit

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P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction 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 Prep Kit (Illumina)
P-1056	EpiNext™ Bisulfite Sequencing 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

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