EpiNext™ DNA Purification HT System

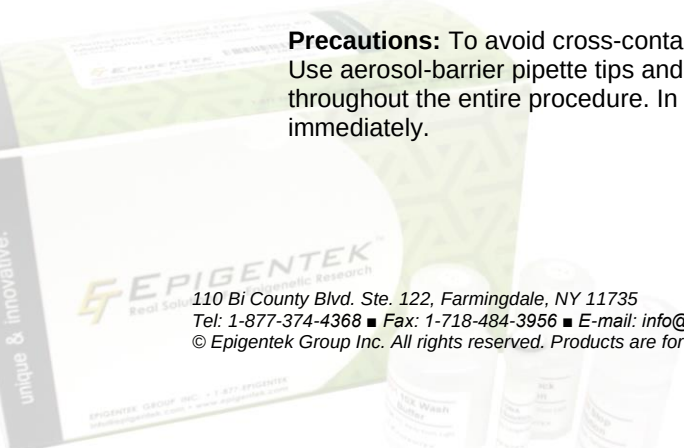
Base Catalog # P-1063

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ DNA Purification HT System utilizes magnetic bead technology for high throughput DNA or PCR amplicon purification and DNA size selection. The system can also be used for concentrating DNA and is suitable for selectively capturing DNA fragments or PCR amplicons that are 100 bps or larger in size. A total of 96 (P-1063-04) or 192 (P-1063-08) standard purifications (use 0.5 µg of DNA in 20 µl of solution), or 48 (P-1063-04) or 96 (P-1063-08) standard DNA size selections (use 50 µl of input DNA solution) can be performed with the bead volume provided in the system.

Starting Material and Input amount: DNA fragments of various lengths. Input amount can be from 0.1 ng to 1 µg.

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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PRODUCT CONTENTS

Component	Cat. #P-1063-04	Cat. #P-1063-08	Cat. #P-1063-64	Cat. #P-1063-X4	Storage Upon Receipt
MQ Binding Beads	4 ml	8 ml	64 ml	480 ml	4°C
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SHIPPING & STORAGE

The product is shipped at room temperature.

Upon receipt: Store the following components at 4°C: **MQ Binding Beads.** Store all other components at room temperature. The beads are stable for at least 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Magnetic stand (96-well format)
- ☐ Pipettes and pipette tips
- ☐ 0.2 ml PCR tubes (for single tube format)
- ☐ 96 well round bottom plate or 96 well cycling plate
- ☐ 80% ethanol
- ☐ DNA sample
- ☐ DNA elution buffer (DNase/RNase-free water or TE buffer)

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ DNA Purification HT System 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 product when using that product.

Usage Limitation: The EpiNext™ DNA Purification HT System is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ DNA Purification HT System and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

Obtaining high recovery of purified DNA or selected DNA fragments is critical for downstream applications that use DNA samples including PCR, sequencing, cloning, microarray, and DNA fragment analysis, regardless of the platform used. The EpiNext™ DNA Purification HT System utilizes magnetic bead technology for high throughput DNA or PCR amplicon purification and DNA size selection. The System has the following features:

- Optimized buffer chemistries: Complete separation of DNA or PCR amplicons. It can also be used for DNA size selection based on the ratio of beads to DNA sample volume.
- Fast and straightforward: The entire procedure can be finished in just 30 min for 96 samples and is highly amenable to a variety of automation platforms. No gels, filtration, centrifugation, or columns are needed.
- Efficient clean-up: Removal of excess primers, adaptors, nucleotides, salts, enzymes, and PCR inhibiting substances, such as polysaccharides, polyphenols, lipids and dyes.
- High recovery of DNA: Higher than 98% recovery of input DNA.
- Manual and automation friendly: Scalable for use in single tube or 96-well plate formats.

PRINCIPLE & PROCEDURE

The EpiNext™ DNA Purification HT System contains an optimized MQ binding bead solution which allows DNA or PCR amplicons to bind tightly to the beads. Excess primers, adaptors, nucleotides, salts, enzymes, and PCR inhibiting substances can be removed by simply washing the beads. Optimization of MQ bead ratio to input DNA allows DNA size selection by the removal of larger or smaller DNA fragments and recovery of desired target size DNA fragments.

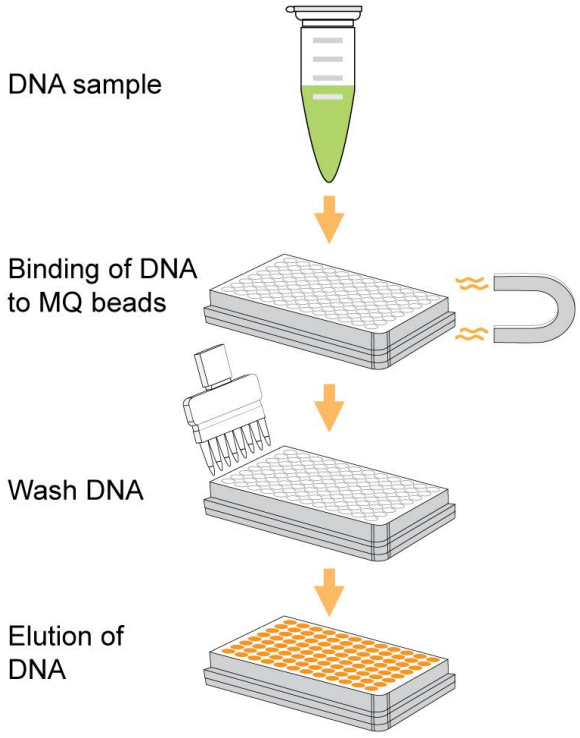

Fig 1. Workflow of the EpiNext™ DNA Purification HT System

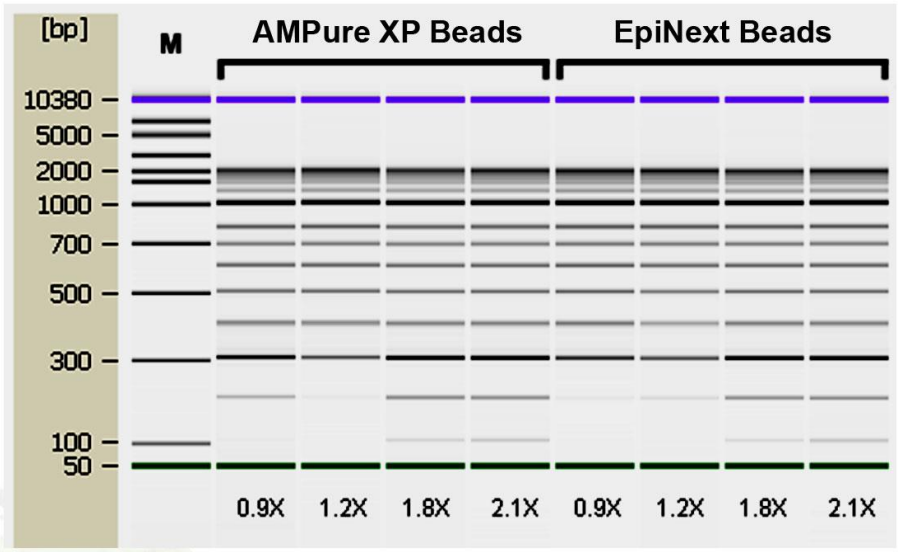

Fig 2. Gel picture after purification of 800 ng of DNA marker (Hyperladder 50 bp ladder, Bioline) using the EpiNext™ DNA Purification HT System with varying ratios of beads from 0.9X to 2.1X. AMPure XP beads were used as the control. M: Marker size of Agilent DNA ChIP kit

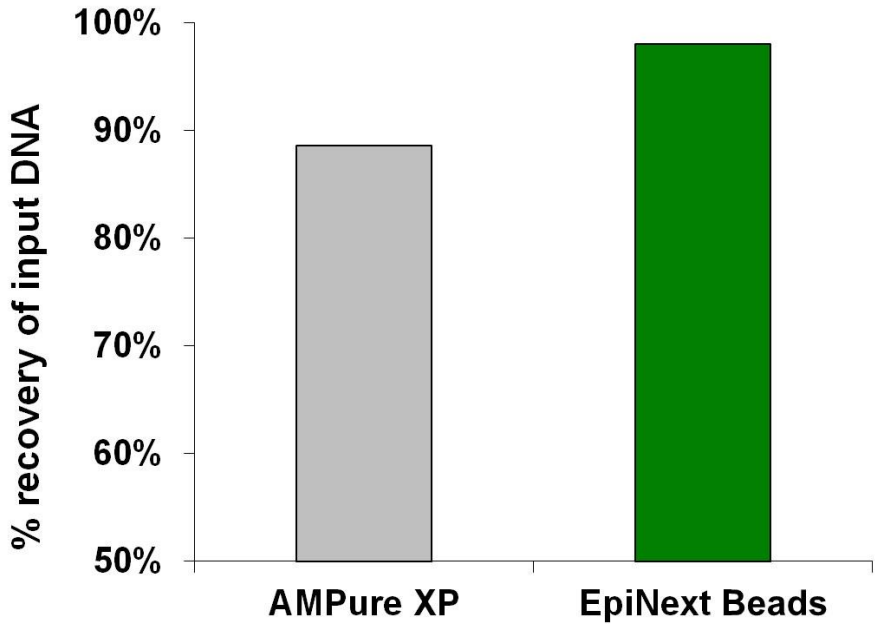

Fig 3. Percentage of recovery of input DNA fragments by "AMPure XP" beads vs "EpiNext" beads: 780 ng of DNA marker (Hyperladder 50 bp ladder, Bioline) were purified using the EpiNext™ DNA Purification HT System at 1.8X. Agencourt AMPure XP beads (1.8X) were used as the control. Recovery yield of 100 bp or higher DNA fragments was quantified with an Agilent Bioanalyzer 2100

ASSAY PROTOCOL

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

Starting Materials

- DNA isolated from various tissues or cell samples: 0.2 ng-1000 ng, optimized 20-500 ng per preparation.
- DNA fragments enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture: 0.2 ng-100 ng.
- cDNA or dsDNA converted from reverse-transcription of RNA or bisulfite-treatment of DNA.
- PCR amplicons.

RNAse I can be used to remove RNA and DNA should be eluted in DNase/RNase-free water.

For the magnetic stand used for capturing DNA bound MQ beads, we recommend using Epigentek's EpiMag™ HT Magnetic Separator, which is very strong and proven to quickly and efficiently achieve high, reproducible retention of magnetic bead-bound DNA in a single PCR tube and in various 96-well plates.

DNA Purification

96 Well Format

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 2X (2:1 ratio) resuspended beads to the DNA sample in 96 well plate (ex: 40 µl of MQ beads to 20 µl of DNA solution). Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to the beads.
d. Put the plate on an EpiMag™ HT Magnetic Separator or an appropriate magnetic stand until the solution is clear (about 2 minutes: if the magnetic stand is not suitable for the plate, transfer the beads solution to an appropriate plate well that is compatible to the magnetic stand). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*
e. Keep the plate in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to each well of the plate. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step e two times for a total of three washes. Make sure that the ethanol is completely removed after the last wash.
g. Air dry beads for 5 minutes at room temperature while the plate is on the magnetic stand 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.*

h. Resuspend the beads in 10-20 µl DNA elution buffer, and incubate at room temperature for 2 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 10-20 µl of supernatant to a new 0.2 ml PCR plate for immediate use or for storage at -20ºC after tightly capping the PCR plate.

Single PCR Tube Format

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 2X (2:1 ratio) resuspended beads to the DNA sample in a 0.2 ml PCR tube (ex: 40 µl of MQ beads to 20 µl of DNA solution). Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to the beads.
d. Put the PCR tube on an EpiMag™ HT Magnetic Separator or 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 to the magnetic stand). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*
e. Keep the tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step e two times for a total of three washes. Make sure that the ethanol is completely removed after the last wash.
g. Air dry beads for 5 minutes at room temperature while the plate is on the magnetic stand 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*.

h. Resuspend the beads in 10-20 µl DNA Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

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

j. Transfer 10-20 µl of supernatant to a new 0.2 ml PCR tube for downstream use or for storage at -20ºC after capping the tube.

Note:** For size selection of DNA fragments, we recommend using the protocol from the EpiNext™ DNA Size Selection Kit (Cat. No. P-1059) which includes the **MQ Binding Beads.

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.	Ensure that RNA is removed by RNAse treatment before starting purification protocol.
	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.
Unexpected peak size of Agilent Bioanalyzer trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected during the DNA library preparation.	Improper ratio of MQ beads to DNA volume during size selection.	Check if the correct volume of **MQ Binding Beads** is added to the DNA solution. Proper ratios should remove the fragments of unexpected peak size.
	Over-amplification of library.	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use proper PCR cycles to avoid this problem.

RELATED PRODUCTS

DNA Isolation and Clean-up

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum 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

Sonication Instruments

EQC-1100	EpiSonic™ Multi-Functional Bioprocessor 1100

DNA Enrichment Reaction

P-1015	Methylamp™ Methylated DNA Capture (MeDIP) Kit
P-1038	EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit
P-1052	EpiQuik™ MeDIP Ultra Kit
P-2002	EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
P-2003	EpiQuik™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2025	ChromaFlash™ One-Step ChIP Kit
P-2026	ChromaFlash™ One-Step Magnetic ChIP kit
P-2027	ChromaFlash™ High-Sensitivity ChIP Kit

PCR Analysis

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-1059	EpiNext™ DNA Size Selection Kit