EpiNext™ DNA Size Selection Kit

Base Catalog # P-1059

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ DNA Size Selection Kit utilizes magnetic bead technology for quick DNA size selection in a high-throughput format. The kit is suitable for removing DNA fragments of <150 bps for DNA library preparation for Illumina, Life Technologies (SOLiD), Ion Torrent and Roche/454 next generation sequencing applications. It can also be used for removing undesired larger DNA fragments by optimizing the bead to DNA volume ratio accordingly. The indicated number of reactions can be performed for a standard 50 µl solution input DNA sample.

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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KIT CONTENTS

Component	48 reactions Cat. #P-1059-48	96 reactions Cat. #P-1059-96	Storage Upon Receipt
MQ Binding Beads*	4 ml	8 ml	4°C
Elution Solution*	1 ml	2 ml	4°C
96-well plate	1	1	RT
User Guide	1	1	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: Store the following components at 4°C: **MQ Binding Beads, Elution Solution.** 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

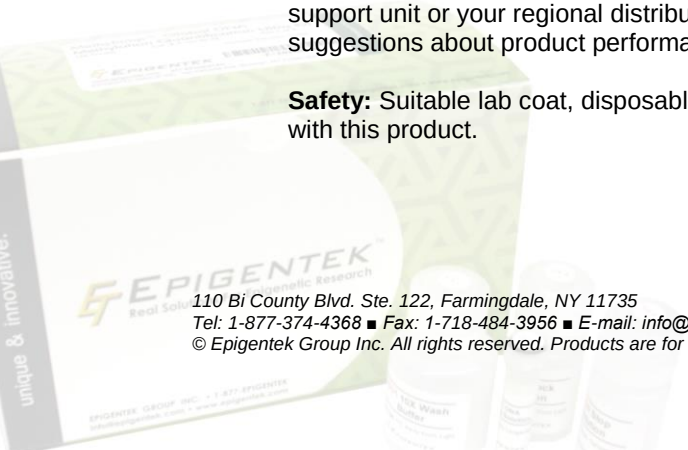
- ☐ Vortex mixer
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of the DNA library
- ☐ 0.2 ml PCR tubes
- ☐ 80% ethanol
- ☐ DNA sample
- ☐ Magnetic stand (96-well format)
- ☐ Pipettes and pipette tips

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ DNA Size Selection 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 EpiNext™ DNA Size Selection Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ DNA Size Selection Kit and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

DNA size selection is necessary after DNA shearing as part of the library construction process for next generation sequencing, regardless of the platform used. Obtaining high recovery of selected DNA fragments is critical for the reduction of sequencing bias. The EpiNext™ DNA Size Selection Kit is optimized for DNA fragment size selection used for various next generation sequencing platforms including Illumina, Life Technologies (SOLiD), Ion Torrent and Roche/454. The EpiNext™ DNA Size Selection Kit has the following features:

- Optimized fragment selection chemistries for complete separation of DNA fragments according to size. Suitable for Illumina, SOLiD, Ion Torrent and Roche/454 platforms.
- Fast and straightforward size selection procedure which can be finished within 30 min. No gels, columns or centrifugation is needed.
- Efficient removal of primer-dimers: No need for further clean-up steps.
- High recovery of targeted DNA fragments: Higher than 85% recovery of input DNA fragments.
- Manual and automation friendly: Scalable for use in single tube or 96-well plate formats

PRINCIPLE & PROCEDURE

The EpiNext™ DNA Size Selection Kit contains all reagents required at each step of the workflow for DNA size selection. The size of DNA fragments bound to MQ Binding Beads is based on the ratio of MQ beads to the DNA sample solution. Optimization of MQ bead ratio to input DNA, allows the removal of larger or smaller DNA fragments and recovery of desired target size DNA fragments.

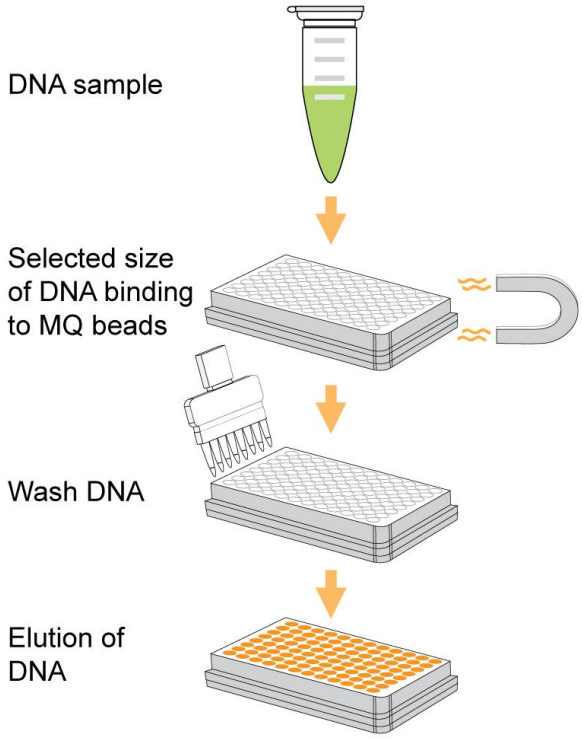


Fig 1. Workflow of the EpiNext™ DNA Size Selection Kit.

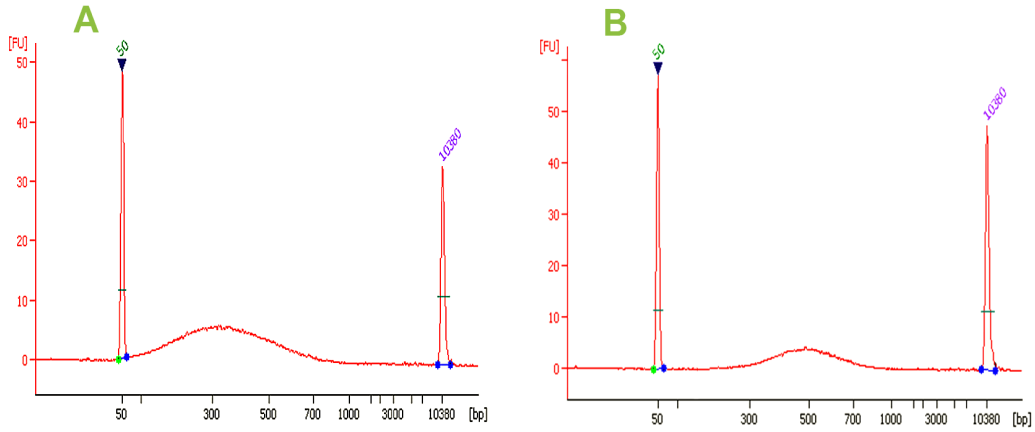


Fig 2. Size selection of DNA fragments. Human Placenta DNA was sheared to 100-700 bps in length with peak size of about 300 bps (Fig A). A target peak size of 500 bps (Fig B) was selected using the EpiNext™ DNA Size Selection Kit.

ASSAY PROTOCOL

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

Starting Materials

Fragmented dsDNA isolated from various tissues or cell samples: 0.2 ng-500 ng, optimized 20-100 ng per preparation.

dsDNA 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.

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

For the magnetic stand used to capture 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.

Size Selection of DNA Fragments

***Note**: If the starting amount of fragmented DNA is less than 50 ng or when large size DNA fragments are not a concern, **Protocol I** can be performed to remove DNA fragments <150 bps (e.g., primers, primer-dimers, adaptors and adaptor-dimers) and for obtaining DNA fragments of 200 bps or larger. Otherwise, **Protocol II** should be used to select desired DNA fragment size (ex: 200-800 bps).*

Protocol I (*For samples containing <50 ng of DNA fragments or when large size DNA fragments are not a concern*)

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 1.2X (1.2:1 ratio) resuspended beads to the DNA sample in a 0.2 ml PCR tube (ex: 60 ul of MQ beads to 50 ul 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 beads.
d. Put the PCR 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 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. Open the tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 10-20 µl **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 PCR amplification.

Protocol II *(For samples containing >50 ng of DNA fragments or that require removal of large size DNA fragments)*

a. Resuspend **MQ Binding Beads** by vortex.
b. Add resuspended **MQ Binding Beads** to the input DNA solution for the desired DNA size according to Table 1, "Beads to DNA ratio in first round selection". Mix well by pipetting up and down at least 10 times.
c. Incubate for 5 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 to 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 resuspended **MQ Binding Beads** to the supernatant for the desired DNA size according to Table 1, "Beads to DNA ratio in second round size selection". 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 min and then carefully remove and discard the ethanol.
h. Repeat Step g one time, for 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.

Table 1. Selection of desired DNA fragment size by optimized ratio of MQ beads to input DNA solution

Desired DNA size (bps)	200	300	400	500	600	800
Beads to DNA ratio in first round selection	0.8X	0.6 X	0.55X	0.5X	0.45X	0.4X
Beads to DNA ratio in second round selection	0.2X	0.2X	0.2X	0.2X	0.2X	0.15X

The quality of the size selected DNA can be assessed by using an Agilent Bioanalyzer or comparable method with or without PCR amplification. If fragments of <150 bp such as primer/adaptor dimers are present in the sample or larger fragments than expected are present in the sample, it is recommend to use 0.8X **MQ Binding Beads** (ex: add 16 µl of **MQ Binding Beads** to 20 µl of sample) to remove them according to **Protocol II** and Table 1 with optimization of the beads to DNA ratio.

The selected DNA fragments can be stored at -20˚C until ready to use.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of selected 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 library preparation 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.	Improper ratio of MQ beads to DNA volume during size selection.	Check if the correct volume of **MQ 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)

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