

MGICare

Detection Set for Chromosome Copy Number Variation Test User Manual

Cat. No.: 1000012919 (96 RXN)

Kit Version: V1.0

Manual Version: 4.0

Revision History

Manual Version	Kit Version	Date	Description
4.0	V1.0	Mar. 2024	<ul style="list-style-type: none"> Update the manufacturer information.
3.0	V1.0	Mar. 2022	<ul style="list-style-type: none"> Update the manufacturer LOGO.
A1	V1.0	Jan. 2021	<ul style="list-style-type: none"> Update contact information.
A0	V1.0	Dec. 2019	<ul style="list-style-type: none"> Initial release.

Note: Please download the latest version of the manual and use it with the corresponding kit.

Search manual by Cat. No. or product name from website:

<https://en.mgi-tech.com/download/files.html>

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Chapter 1 Product Description

1.1 Introduction

MGICare Detection Set for Chromosome Copy Number Variation Test is specifically designed for MGI high-throughput sequencing platforms. The set can prepare libraries with 50 ng of genomic DNA extracted from human tissue, amniotic fluid, umbilical cord blood, peripheral blood or villus and can be used for chromosomal copy number variation detection. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Applications

This library prep kit is applicable for genomic DNA extracted from fetal tissue, amniotic fluid, umbilical cord blood, peripheral blood, villi, etc.

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500RS (SE 50)

MGISEQ-2000RS (SE 50) / DNBSEQ-G400RS(SE 50)

MGISEQ-200RS (SE 50) / DNBSEQ-G50RS (SE 50)

1.4 Contents

MGICare Detection Set for Chromosome Copy Number Variation Test can be used for up to 96 reactions. Further information of Cat No., Components, and Specifications are listed below:

Table 1 MGIcare Detection Set for Chromosome Copy Number Variation Test (96 RXN) (Cat. No: 1000012919)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec & Quantity
MGIcare Detection Kit for Chromosome Copy Number Variation Test (Box 1) Cat. NO. : 1000005290	Fragment and ERAT Buffer Mix	Neutral	720 μ L/tube \times 1tube
	Fragment and ERAT Enzyme Mix	Neutral	240 μ L/tube \times 1tube
	Ligation Buffer Mix	Red	1584 μ L/tube \times 2 tubes
	DNA Ligase	Red	192 μ L/tube \times 1tube
	PCR Enzyme Mix	Blue	1200 μ L/tube \times 2 tubes
	PCR Primer Mix	Blue	192 μ L/tube \times 1tube
	CNV Control-1	Yellow	10 μ L/tube \times 1tube
	CNV Control-2	Yellow	10 μ L/tube \times 1tube
MGIcare Detection Kit for Chromosome Copy Number Variation Test (Box2) Cat. NO. : 1000005290	Barcode Adapter Mix(01-96)	/	18 μ L/well \times 96 wells
MGIcare Detection Kit for Chromosome Copy Number Variation Test (Box 3) Cat. NO. : 1000005290	DNA Clean Beads	White	4320 μ L/tube \times 2 tubes
	TE Buffer	White	5088 μ L/tube \times 1tube
	Molecular Water	White	1920 μ L/tube \times 1tube
MGIeasy Rapid Circularization Module Cat. NO. : 1000005258	Splint Buffer	Purple	186 μ L/tube \times 1tube
	DNA Rapid Ligase	Purple	8 μ L/tube \times 1tube

1.5 Storage Conditions and Shelf Life

MGICare Detection Kit for Chromosome Copy Number Variation Test (Cat. No. : 1000005290) Box 1

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to label.
- Transportation Conditions: transported in dry ice.

MGICare Detection Kit for Chromosome Copy Number Variation Test (Cat. No. : 1000005290) Box 2

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to label.
- Transportation Conditions: transported in dry ice.

MGICare Detection Kit for Chromosome Copy Number Variation Test (Cat. No. : 1000005290) Box 3

- Storage Temperature: 2°C to 8°C.
- Production Date and Expiration Date: refer to label.
- Transportation Conditions: transported in ice packs

MGIEasy Rapid Circularization Module (Cat. NO.: 1000005258)

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to label.
- Transportation Conditions: transported in dry ice.

* Please ensure that an abundance of dry ice remains after transportation.

* Performance of products are guaranteed until the expiration date under appropriate transport, storage, and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 2 Equipment and Materials Required but not Provided

Equipment	Vortex Mixer Desktop Centrifuge Pipets Thermocycler Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Qubit™ 3 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216) Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA)
Reagents	100% Ethanol (Analytical Grade) Qubit™ ssDNA Assay Kit (Thermo Fisher Scientific™, Cat. No. Q10212) Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific™, Cat. No. Q32854) DNA Analysis Kits (Agilent Technologies™, Cat. No. 5067-1504) MGISEQ or BGISEQ or DNBSEQ High-throughput Sequencing Set
Consumables	Pipette Tips 1.5 mL EP tube, 1.5 mL MaxyClear Snaplock Microcentrifuge Tube (Axygen™, Cat. No. MCT-150-C) or equivalent Axxygen™ 0.2 mL Thin Wall PCR Tubes (Axygen™, Cat. No. PCR-02-C) or Axxygen™ 96-well Polypropylene PCR Microplate (Axygen™, Cat. No. PCR-96M2-HS-C) Qubit™ Assay Tubes (Thermo Fisher Scientific™, Cat. No. Q32856) or Axxygen™ 0.5 mL Thin Wall PCR Tubes (Axygen™, Cat. No. PCR-05-C)

1.7 Precautions and Warning

- This product is for scientific research only and is not intended for clinical diagnosis. Please read this manual carefully before use.
- To prevent cross contamination, we recommend using filtered Pipette Tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermocyclers with heated lids for reactions. Preheat to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR products and may negatively affect experimental accuracy. We recommend physically separating two working areas in the laboratory for PCR reaction preparation and PCR product cleanup. Use designated equipment for each area and perform regular cleaning to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment).
- If you have other questions, contact Technical Support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation

The MGIcare Detection Kit for Chromosome Copy Number Variation Test can be used to prepare libraries using genomic DNA extracted from human fetal tissue, amniotic fluid, umbilical cord blood, peripheral blood, or villus. Genomic DNA must meet the following four criteria:

- The amount of DNA is at least 50 ng.
- The DNA concentration is at least 2.5 ng/ μ L.
- High DNA purity ($OD_{260}/OD_{280}=1.8$ to 2.0).
- DNA integrity should be high quality or minimally degraded genomic DNA. The requirement of DNA integrity in this kit, agarose gel electrophoresis results are as follow:

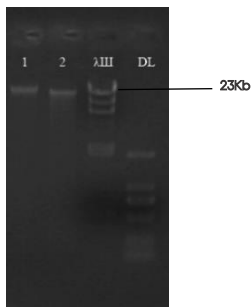


Figure 1 Agarose gel electrophoresis result of genomic DNA*

*Sample 1 is high quality genomic DNA, sample 2 is minimally degraded genomic DNA



Note: Genomic DNA with high integrity is suggested for the library preparation (high quality genomic DNA exhibits as a single band larger than 23 kb without smear in the 1% agarose gel electrophoresis). Although high quality genomic DNA means high library success rate and reliable sequencing data, slightly degraded genomic DNA is still acceptable for library preparation when high integrity genomic DNA is inaccessible. It is recommended to use highly purified genomic DNA. Leftover proteins, salts, or other contaminants in the DNA can suppress enzyme activity.

Chapter 3 Library Construction Protocol

3.1 Reagent Preparation

Thaw buffers at room temperature before use. Vortex to mix and briefly centrifuge, then place on ice. Warm DNA Clean Beads, molecular-grade water and TE buffer to room temperature. Precipitates may appear when the buffer has thawed but this not affect the functionality of the buffer. Vortex to mix until the precipitate disappears before use.

3.2 DNA Fragmentation and End Repair

- 3.2.1 Transfer 50 ng gDNA to a new PCR tube. Add enough molecular grade water to the tube to bring the total volume to 20 μL . Mix the tube thoroughly and centrifuge briefly.



Note: Sample Volume (μL) = 50 ng / concentration of gDNA (ng/ μL); The concentration of CNV Control-1 and CNV Control-2 is 10 ng/ μL respectively.



Note: CNV detection result of CNV Control-1 is trisomy 21; CNV detection result of CNV Control-2 is negative for above 1M.

- 3.2.2 Prepare the following fragmentation and end repair reaction mixture in a new microfuge tube (see Table 3).

Table 3 Fragmentation and End Repair Reaction Mixture

Components	Volume
Fragment and ERAT Buffer Mix	7.5 μL
Fragment and ERAT Enzyme Mix	2.5 μL
Total	10 μL

- 3.2.3 Add 10 μL of the Fragmentation and End Repair Reaction Mixture to the PCR tube prepared in step 3.2.2, Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.
- 3.2.4 Place the 0.2 mL PCR tube from step 3.2.3 into the thermocycler and run the program in Table 4

Table 4 Fragmentation and End Repair Reaction Conditions

Temperature	Time
Heated lid	On
37°C	15 min
65°C	15 min
4°C	Hold

3.2.5 Briefly centrifuge to collect the solution at the bottom of the tube.



Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.3.

3.3 Adapter Ligation



Note: Please refer to appendix A for detailed adapter combination instructions.

3.3.1 Add 15 μL of Barcode Adapter Mix (01-96) (one barcode adapter for each sample) to the PCR tube from step 3.2.5. Vortex 3 times (3s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.3.2 Prepare the following ligation reaction mixture in a new microfuge tube (see Table 5).

Table 5 Ligation Reaction Mixture

Components	Volume
Ligation Buffer	33 μL
DNA Ligase	2 μL
Total	35 μL

3.3.3 Pipette slowly to transfer 35 μL of Adapter ligation mixture to the 0.2 mL PCR tube from step 3.3.1. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.3.4 Place the PCR tube from step 3.3.3 into the thermocycler and run the program in Table 6.

Table 6 Ligation Reaction Conditions

Temperature	Time
Heated lid	On
23°C	20 min
4°C	Hold

3.3.5 Centrifuge briefly to collect the solution at the bottom of the tube and transfer the solution to a new microcentrifuge tube.



Note: Please perform step 3.4 immediately after step 3.3. Otherwise, the yield may decrease.

3.4 Cleanup of Adapter-ligated DNA



Note: Read appendix B carefully before the purification.

- 3.4.1 Take out DNA Clean Beads from refrigerator and bring to room temperature for at least 30 min. Vortex and mix thoroughly before use.
- 3.4.2 Transfer 40 μ L DNA Clean Beads to the tube in step 3.3.5. Mix gently by pipetting up and down at least 10 times. Ensure that all of the liquid is fully dispensed from the pipette tip into the tube before proceeding.
- 3.4.3 Incubate the tube at room temperature for 5 min.
- 3.4.4 Centrifuge briefly and place the tube onto a magnetic separation rack for 2-5 min until the supernatant is clear. With the tube on the magnetic separation rack, remove and discard the supernatant.
- 3.4.5 Keep the tube on the magnetic separation rack, add 200 μ L freshly prepared 80% ethanol to wash the beads and the sides of the tube. Incubate 30 s then carefully remove and discard the supernatant.
- 3.4.6 Repeat step 3.4.5 once, and remove all supernatant from the tube without disrupting the beads. Briefly centrifuge the tube and return it to the magnetic separation rack. Carefully remove and discard the remaining supernatant using a small volume pipette.
- 3.4.7 Keep the tube on the magnetic separation rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed. Avoid over-drying beads (cracks can be observed on pellet).
- 3.4.8 Remove the tube from the magnetic separation rack, add 25 μ L TE Buffer. Pipette the mixture at least 10 times to re-suspend the beads.
- 3.4.9 Incubate at room temperature for 5 min.
- 3.4.10 Centrifuge briefly and place the tube back onto the magnetic separation rack for 2-5 min until beads the supernatant is clear. Transfer 23 μ L supernatant to a new PCR tube.



Stopping Point: After cleanup, adapter-ligated DNA can be stored at -20°C

3.5 PCR Amplification

- 3.5.1 Prepare the following PCR reaction mixture in a new microfuge tube (see Table 7).

Table 7 PCR reaction mixture

Components	Volume
PCR Reaction Buffer	25 μ L
PCR Primer Mix	2 μ L
Total	27 μ L

- 3.5.2 Add 27 μ L PCR reaction mixture to the PCR tube prepared in step 3.4.10, then Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.
- 3.5.3 Place the PCR tube from 3.5.2 into the thermocycler and run the program in Table 8.

Table 8 PCR Reaction Conditions

Temperature	Time	Cycles
Heated Lid	on	
95°C	3 min	1 cycle
98°C	15 s	
56°C	15 s	7 cycles
72°C	30 s	
72°C	5 min	1 cycle
4°C	Hold	

- 3.5.4 After the program has completed, briefly centrifuge the tube.

3.6 Cleanup of PCR production



Note: Read appendix B carefully before the purification.

- 3.6.1 Take out DNA Clean Beads from refrigerator and incubate at room temperature for at least 30 min beforehand. Vortex and mix thoroughly before use.
- 3.6.2 Transfer 35 μ L DNA Clean Beads to the tube in step 3.5.4. Mix by pipetting up and down at least 10 times. Ensure that all of the liquid is fully dispensed from the pipette tip into the tube before proceeding.
- 3.6.3 Incubate the tube at room temperature for 5 min.
- 3.6.4 Centrifuge briefly and place the tube back onto the magnetic separation rack for 2-5 min

until the supernatant is clear. With the tube on the magnetic separation rack, transfer the supernatant to a new microfuge tube.



Note: Reserve the supernatant and discard the DNA Clean beads in this step.

- 3.6.5 Transfer 15 μ L DNA Clean Beads to the tube in step 3.6.4. Mix by pipetting up and down at least 10 times. Ensure that all of the liquid is fully dispensed from the pipette tip into the tube before proceeding.
- 3.6.6 Incubate at room temperature for 5 min.
- 3.6.7 Centrifuge briefly and place the tube back onto a magnetic separation rack for 2-5 min until the supernatant is clear. With the tube on the magnetic separation rack, carefully remove and discard the supernatant.
- 3.6.8 Keep the tube on the magnetic separation rack and add 200 μ L freshly prepared 80% Ethanol to wash the beads and the sides of the tube. Incubate for 30s, then carefully remove and discard the supernatant.
- 3.6.9 Repeat step 3.6.8 once and remove all supernatant from the tube without disrupting the beads. Briefly centrifuge the tube and return it to the magnetic separation rack. Carefully remove and discard the remaining supernatant using a small volume pipette.
- 3.6.10 Keep the tube on the magnetic separation rack with the lid open to air-dry the beads until no wetness (reflectiveness) is observed. Avoid over-drying beads (cracks can be observed on pellet).
- 3.6.11 Remove the tube from the magnetic separation rack and add 25 μ L TE Buffer. Pipette the mixture at least 10 times to re-suspend the beads.
- 3.6.12 Incubate at room temperature for 5 min.
- 3.6.13 Centrifuge Briefly and place the EP tube back onto the magnetic separation rack for 2-5 min until beads pellet and the supernatant is clear. Transfer 23 μ L supernatant to a new 1.5 mL tube.



Stopping Point: After cleanup, adapter-ligated DNA can be stored at -20°C

3.7 Quality Control of PCR Products and ssDNA Circularization

- 3.7.1 Quantitate the PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is at least 2 ng/μL.
- 3.7.2 One to two PCR products are selected for each batch of experiments to assess the fragment size distribution of PCR products with electrophoresis based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer) or Fragment Analyzer™ (Advanced Analytical). The peak of the PCR products should be between 200 to 300 bp.

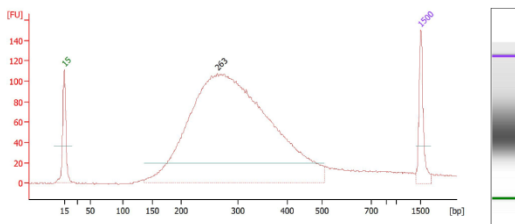


Figure 2 The Agilent 2100 Bioanalyzer Result of PCR Product

- 3.7.3 If more than one samples are pooled for sequencing, please read the detailed information in Appendix A carefully before pooling the sample. Briefly, PCR libraries with different adapters are mixed in equal amounts to a total of 170 ng. Add TE Buffer until the final volume is 48 μL, mix by vortexing, and centrifuge briefly.
- 3.7.4 The mixed PCR libraries can be stored at -20°C. Otherwise, continue to the ssDNA circularization step. Circularized ssDNA is compatible with MGI high-throughput sequencing platforms, and can be prepared with the 'MGIEasy Rapid circularization module' (Cat. No.: 1000005258).
- 3.7.5 To prepare circularized ssDNA, incubate the pooled PCR libraries from 3.6.13 at 95°C for 3 min in a PCR thermocycler, then cool the PCR tubes on the ice for 2 min.
- 3.7.6 Prepare the following PCR reaction mixture in a new microfuge tube (see Table 9).

Table 9 Circularization Reaction Mixture

Components	Volume
Splint Buffer	11.6 μ L
DNA Rapid Ligase	0.5 μ L
Total	12.1 μ L

- 3.7.7 Add the 12.1 μ L of circularization reaction mixture into each of the PCR tubes from 3.5.4. Mix by vortexing, centrifuge briefly, and incubate the sample at 37°C for 30 min in the thermocycler. Centrifuge briefly after the reaction.

✓ **Stopping Point: the circularized ssDNA can be stored at -20°C**

Chapter 4 DNB making and Sequencing

About 20 μ L of circularized ssDNA product are used to prepare DNA nanoballs (DNBs) and sequencing.

Please follow the protocol described in "BGISEQ/MGISEQ/DNBSEQ High-throughput Sequencing Set Instruction Manual" for DNB making and sequencing. The available sequencing kits including:

BGISEQ-500RS sequencing platform: SE50

MGISEQ-2000RS \ DNBSEQ-G400RS sequencing platform: SE50

MGISEQ-200RS \ DNBSEQ-G50RS sequencing platform: SE50

Appendix

Appendix A Barcode Adapter Mix (01-96) user manual

Based on the principles of balanced base composition, Adapters must be used in specific groups. Please follow the instructions below to use Adapters in proper combination.

4 sets of 4 Adapters: Column 1-2 (01-04, 05-08, 09-12, 13-16)

10 sets of 8 Adapters: Column 3-12 (17-24, 24-32, 3-40, 41-48, 49-56, 57-64, 65-72, 73-80, 81-88 and 89-96)

If the sequencing data output requirement is the same for all samples in a lane, please refer to the Table 10 below to organize your barcode Adapter combinations.

Table 10 Adapters 01-96 (Plate) Kit Instruction

Sample/lane	Instruction (Example)
1	<p>1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample.</p> <p>Or 2. Take a set of 8 Adapters (e.g. 41-48), mix 8 Adapters with equal volumes, then add the mixture to the sample.</p>
2	<p>1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2)</p> <p>Or 2. Take a set of 8 Adapters (41-48), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 41-44, then add to sample 1; Mix 45-48, then add to sample 2)</p>
3	<p>For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for sample 1,2 and 3.</p>
4	<p>1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Respectively add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4.)</p> <p>Or 2. Take a set of 8 Adapters (41-48), mix Adapters with equal volumes in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 41-42, 43-44, 45-46, 47-48, then add respectively to samples 1, 2, 3, 4.)</p>
5	<p>For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the</p>

	method for (1 sample/lane) above. Note that you should use different Adapter sets for sample 1-4 and 5.
6	For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for sample 1-4 and 5-6.
7	1) For samples 1-4, use the method for (4 samples/lane) above. (Use 1st Adapter set) 2) For samples 5-6, use the method for (2 samples/lane) above. (Use 2nd Adapter set) 3) For sample 7, use the method for (1 sample/lane) above. (Use 3rd Adapter set) Note that you should use different Adapter sets for sample 1-4, sample 5-6 and sample 7.
8	Take a set of 8 Adapters (e.g. 41-48), add 1 Adapter to each sample in an equal volume.
8n+x (n=1,2, x=1-8, Total 9-24)	Follow these 3 steps: 1) For samples 1-8, use the method for (8 samples/lane) above. Or separate into 2 groups of 4 and use the method for (4 samples/lane) above for each group. 2) For samples 9-8n, separate samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).
8n+x (3≤n<11, x=1-8, Total 25-96)	Follow these 3 steps: 1) For samples 1-24, take a set of 24 Adapters and add 1 Adapter for each sample in an equal volume. 2) For samples 25-8n, separate the samples into groups of 8, and use the method for (8 samples/lane) above. 3) For samples 8n+1 - 8n+X, according to the value of X, use the methods above for 1-8 sample/lane accordingly. Remember to use different Adapter sets. Note that you should use different Adapter sets for steps 1), 2) and 3).

For situations in which sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters. For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (89-96), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

Appendix B DNA Clean Beads and Cleanup Procedures

Before You Use

- To ensure capture efficiency of the DNA Clean Beads, remove beads from 4°C refrigerator storage, and equilibrate to room temperature before use. Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional TE buffer to the designated volume before using the beads to purify. It ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 minutes. Consider the different magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear.
- Avoid touching the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In the event of contact between the beads and the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads. Keep the centrifuge tube on the Magnetic Separation Rack when washing with ethanol. Do not shake or disturb the beads in any way.
- After the 2nd wash of beads with ethanol, try to remove all of the liquid from the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically, and remove the remaining liquid by using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 minutes depending on your specific lab environment. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.
- During the elution step, do not touch the beads with the pipette tips when removing the supernatant. Contamination in DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2 μL more than the volume of the

supernatant.

- Pay attention when opening/ closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

Contact Us

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