AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® DNA Normalization Kit

Version 1.2

Magnetic bead-based kit for normalization of DNA concentration, and quantitation of DNA for NGS and other applications

Catalog Number: S006-00, S006-01, S006-02,

Contents

Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

The Auto-Mag® DNA Normalization Kit is a paramagnetic bead-based kit. DNA normalization is based on the binding of DNA to proprietary beads of limited binding capacity; excess DNA is washed away, and a normalized amount of DNA is recovered. With this kit, the required uniform DNA concentration can be obtained from DNA samples of various concentrations without considering the concentration of the initial input DNA during the DNA normalization process, and without using fluorescence measurement or other methods; thus, saving time and operating costs. This protocol does not require a centrifugation step and can be performed in 96-well plates (manual and automated) and single tubes (manual only) using related protocols.

The kit also contains Auto-Mag® PCR-Pure reagent, which can be used in the recovery of unbound excess DNA during DNA normalization. Auto-Mag® PCR-Pure also can be used for PCR/DNA purification, or DNA fragment size selection for NGS library preparation. For more detail information, please reference Auto-Mag® PCR-Pure (Cat #: S002)

Features

- Rapid and reliable quantitation and normalization of DNA
- No centrifugation step, no filtration step
- Allowing the processing of pool DNA samples from various sources
- Equalizing input genomic DNA concentration for DNA library construction to help produce consistent and reliable NGS data without tedious initial input DNA quantitation.
- Reduce library construction time, reagents usage and overall costs

Kit Contents

Product Number	S006-00	S006-01	S006-02
Number of Preps*	5	50	200
Auto-Mag® C-7	0.06 ml	0.6 ml	2.2 ml
NC Buffer	0.5 ml	5 ml	20 ml
Auto-Mag® PCR-Pure	0.5 ml	5 ml	20 ml
Elution Buffer	1 ml	10 ml	40 ml
*Number of reactions is based on 50µl gDNA sample.			

Storage and Stability

Auto-Mag® DNA Normalization Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly: Auto-Mag® C-7 can be stored at room temperature (15-25°C) for 12 months, to prolong the shelf-life, storage at 2-8°C is recommended. Auto-Mag® PCR-Pure should be stored at 2-8°C, and all other components can be stored at room temperature.

During shipment or storage in cool ambient conditions, the precipitates may form in some buffers. Check buffers and re-dissolve any precipitates by warming the buffer at 37˚C. and gently shaking before using.

Preparation of Reagents

1. Prepare 80% ethanol for wash steps. A minimum of 0.6 ml is required per sample.

Additional Information

1. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	DNA, PCR amplicon
Starting Amount	50 µl
Binding capacity	Scalable
Downstream Application	NGS
Elution Volume	25 µl or above
Processing format	Automated; Manual
Storage	2°C - 8°C

Auto-Mag® gDNA Normalization Protocol

The gDNA normalization is based on the magnetic beads and AMD's proprietary binding buffer. The amount of the Auto-Mag® C-7 is optimized to have a limited binding capacity, thus using same volume of Auto-Mag® C-7 in sample will recovery an equal amount of gDNA. In this protocol, the standard protocol of gDNA normalization will recover about 400ng gDNA from 50µl of gDNA sample.

Although any amount gDNA can be used in normalization, the binding capacity of the bead varies with the size and source of DNA. The amount of gDNA that will bind to the beads depends on the efficiency of the extraction protocol, quality, and quantity of the starting materials. For the best results, we highly recommend the user to quantify a few samples to find normalization range of your samples.

Additionally, Auto-Mag® DNA Normalization Kit is not for direct extraction of gDNA from cells or tissues. Adding less gDNA than the recommended input amount may also cause more variation in the normalized product. The recommended input amount of total gDNA is 1000ng or greater in 50µl samples. gDNA that does not bind to the Auto-Mag® C-7 can be recovered with Auto-Mag® PCR-Pure reagents by using the unbound DNA recovery protocol.

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.5-2.0 ml microcentrifuge tube, and Magnetic Rack Separator for microcentrifuge tube.
- 96-well format96-well format: 96-well plate with a >250 capacity and compatible magnetic separation device
- Multichannel pipette
- Polypropylene reservoirs
- 100% Isopropanol
- 80% Ethanol

Before Starting

- Prepare 80% ethanol for wash steps. A minimum of 0.6 ml is required per sample.
- Complete resuspension of the Auto-Mag® C-7 by vortex and keep at room temperature for at least 30 min before use.

Protocol for 96-well Plate Format

1. Transfer 50µl gDNA sample to be normalized to a well of 96-well plate. If the sample volume is less than 50µl, adjust the DNA volume to 50µl with Elution Buffer or Nuclease-Free Water. Do not change the reaction size. Label the plate as sample plate.

2. Add 50µl NC Buffer and 10µl Auto-Mag® C-7 to each sample well. Mix well by pipetting 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes.

   Note: Recommendation: For use with multiple samples, Prepare master mixture of NC Buffer / Auto-Mag® C-7: (50ul NC buffer, 10ul Auto-Mag® C-7 for one sample) and add 60ul mixture to each sample well.

3. Place the sample plate on a compatible magnetic separation device for 3-5 minutes or until the magnetic beads are completely cleared from solution. Remove all the supernatants. Do not disturb the attracted beads.

   Note: If unbound DNA needs to be recovered, transfer supernatant to a new 96-well plate. Label as DNA recovery plate. Refer to the "Unbound DNA Recovery" protocol.

4. With the samples plate on the magnet, add 200µl of 80% ethanol to each sample and incubate for 30 seconds at room temperature. Remove and discard all of liquid. Do not disturb the attracted beads.

   Note: It is not necessary to resuspend the Auto-Mag® C-7.

5. Repeat Steps 4 for a second and third 80% Ethanol rinse step.

6. Keep the sample plate on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

   Note: It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

7. Remove the sample plate from the magnet. Add 25µl Elution Buffer to each sample and mix by pipetting 20 times.

8. Seal the plate and incubate at 65ºC for 5 minutes.

9. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads are completely cleared from solution.

10. Transfer the eluate containing the normalized DNA to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.

    Note: Do not carry over any Auto-Mag® C-7 beads when transferring the eluate.

Auto-Mag® PCR Amplicon Normalization Protocol

This protocol allows for high-throughput PCR amplicon clean-up and normalization. The standard process involves first adding 3 volumes of NC buffer to the original PCR reaction product or purified PCR Amplicon and add 10ul the Auto-Mag® C-7 to the mixture. The bound PCR amplicon is then washed with 80% ethanol (not supply) to remove any remaining impurities, and the purified PCR amplicon is eluted with Elution Buffer. The purified and normalized PCR amplicon can then be used in NGS workflow and other sequencing applications.

Adding less DNA than the recommended input amount will cause more variation in the normalized product. The recommended DNA input amount must be 400 ng or greater. Since PCR products have different sizes or different reaction buffer and volume, it is highly recommended to users to establish a method based on each DNA fragment size of interest to expect less variability in the desired DNA output. Generally, 200bp-1000bp DNA fragments show similar response.

Materials and Equipment to be supplied by User:

- 96-well PCR plate with a capacity of >250ul and compatible with the magnetic separation device used Multichannel pipette
- Polypropylene reservoirs
- Sealing film
- Magnetic separation device compatible with 96-well PCR plate
- 100% Ethanol (do not use denatured ethanol)
- 80% Ethanol

Before Starting

- Prepare fresh 80% Ethanol, (Prepare from absolute ethanol. Do not use denatured alcohol).
- Thoroughly shake the Auto-Mag® C-7 to resuspend the beads before use.

Protocol for 96-well Plate Format

1. Confirm the PCR sample reaction Volume after finishing PCR reactions.

2. For 25µl PCR sample, add 75µl NC buffer, and 10µl Auto-Mag® C-7.

Note: If the sample volume is not 25µl, but the sample volume is between 20µl-50µl, Add 3 volumes of NC buffer directly to each well containing the PCR reaction products. Do not adjust the volume of Auto-Mag® C-7

added to the reaction as it will increase variation.

3. Mix thoroughly the Auto-Mag® C-7 and sample by pipetting up and down 10 times. Incubate the mixture at room temperature for 5 minutes.

4. Place the sample plate on the magnetic separation device for 3-5 minutes or until the Auto-Mag® C-7 beads are completely cleared from solution. Carefully remove and discard the supernatant.

5. Keep the sample plate on the magnetic separation device and add 200µl 80% Ethanol to each sample and incubate for 1 minute at room temperature. Carefully remove and discard the supernatant.

   Note: It is not necessary to resuspend the Auto-Mag® C-7.

6. Repeat Steps 5 for a second and third 80% Ethanol rinse step.

7. Keep the sample on the magnetic separation device and allow the sample to air-dry at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

   Note: It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

8. Remove the sample plate from the magnetic separation device. Add 25µl Elution Buffer to each sample and mix by pipetting up and down 20 times.

9. Seal the plate and incubate at 65ºC for 5 minutes.

10. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the Auto-Mag® C-7 clear from solution.

11. Transfer the eluate containing the normalized DNA to a new plate.

    Note: Do not carry over any Auto-Mag® C-7 when transfer the elution.

12. Store the normalized gDNA at -20°C.

Protocol for Unbound gDNA Recovery

The following protocol is for recovery unbound gDNA after gDNA normalizing procedure. There are up to about 4000ng gDNA can be recovered from supernatant.

Materials and Equipment to be supplied by User:

- 96-well PCR plate with a capacity of >500ul and compatible with the magnetic separation device used Multichannel pipette
- Polypropylene reservoirs
- Sealing film
- Magnetic separation device compatible with 96-well PCR plate
- 100% Ethanol (do not use denatured ethanol)
- 80% Ethanol

Before Starting

- Prepare 80% ethanol for wash steps. A minimum of 0.5 ml is required per sample.
- Thoroughly shake Auto-Mag® PCR-Pure reagents to resuspend the beads before use.

Procedure

1. Complete Steps 1-4 of the gDNA Normalization protocols before beginning this protocol.
2. Transfer the supernatant to a new 96-well PCR plate. Label as DNA recovery plate.
3. Confirm the volume of the supernatant. Add same volume of *Auto-Mag*® PCR-Pure reagents to the sample. (For example: For 100µl the supernatant, add 100µl Auto-Mag® PCR-Pure reagents).
4. Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting up and down 10 times. Incubate the mixture at room temperature for 5 minutes.
5. Place the DNA recovery plate on the magnetic separation device for 3-5 minutes or until the Auto-Mag® PCR-Pure beads are completely cleared from solution. Carefully remove and discard the supernatant.
6. Keep the DNA recovery plate on the magnetic separation device, add 300µl 80% Ethanol to each sample and incubate for 1 minute at room temperature. Carefully remove and discard the supernatant.

Note: It is not necessary to resuspend the Auto-Mag® PCR-Pure reagents

7. Repeat step 6 for a second 80% ethanol wash.

8. Leave the DNA recovery plate on the magnetic separation device and allow the sample to air-dry at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

9. Remove the DNA recovery plate from the magnetic separation device. Add 25-100µl Elution Buffer to each sample and mix by pipetting up and down 15 times.

10. Incubate DNA recovery plate at room temperature for 5 minutes.

Note: Prewarming the elution buffer at 65°C can increase the yield.

11. Place the recovery plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads are completely cleared from the solution.

12. Transfer the eluate containing the recovered DNA to an appropriate storage vessel and keep at -20°C for long term storage.

Note: Do not carry over any Auto-Mag® PCR-Pure reagents when transfer the elution.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via Phone: 1-404-290-5063 (in US), Email: support@amdbiotech.com

Observation	Possible Causes	Comments
Low gDNA Yields	Low Input DNA	Use at least 2000 ng gDNA to achieve desired results.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® DNA Normalization Kit	S006-00 S006-01 S006-02 S006-03	5 Preps. 50 Preps. 200 Preps. 2000 Preps.

Related Products and Reagents

Product Description	Catalog No.	Size
Auto-Mag® PCR-Pure	S002-01 S002-02 S003-03 S003-04	5 ml 50 ml 500 ml 1000 ml

AMD Biotech is committed to simplifying your research with quality products and services.

If you are dissatisfied with this product for any reason, please call 404-290-5063

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