Auto-Mag® DNA Normalization Kit

Version 2.2

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, S006-01P, S006-02P

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.

Protocol Modification Note:

In version 2.2 of the standard protocol for normalizing gDNA or PCR amplicons, the elution buffer volume is increased from 25µL to 40µL. This adjustment simplifies calculations and enables more precise aliquoting of DNA for applications requiring less than 400 ng, without changing the core steps or modifying the protocol. Additionally, the increased elution buffer volume reduces pipetting errors. All other procedural steps and requirements remain unchanged from version 2.1."

Product Introduction

The Auto-Mag® DNA Normalization Kit is a paramagnetic bead-based solution designed to ensure quick and consistent recovery of a predetermined amount of DNA, even from samples with varying input levels. It is compatible with a wide range of DNA types, including genomic DNA, PCR amplicons, DNA fragments, and NGS libraries.

The core of the kit is its innovative magnetic bead technology, which has a limited DNA binding capacity. Once the beads are saturated with target DNA from the sample, any excess unbound DNA is separated and washed away, allowing for the recovery of a precise amount of DNA. Following standard operating procedures, typical normalized yields are approximately 400 ng for genomic DNA or PCR amplicon, and approximately 100 ng for DNA fragments (200-500 bp) or NGS library products. The kit also provides flexibility, enabling users to adjust the volume of magnetic beads or volume of elution buffer to meet specific requirements for DNA yield or concentration. The normalized DNA is ready for a variety of applications, including PCR, sequencing, NGS library preparation, and other molecular biology workflows.

Traditional DNA normalization workflows often require a standard curve with DNA samples of known concentration, a process that can be labor-intensive and reagent-heavy. The Auto-Mag® DNA Normalization Kit simplifies this, delivering the expected DNA recovery in just three simple steps: bind, wash, and recover. It offers a reliable, efficient, and cost-effective solution for researchers seeking to streamline DNA normalization while maintaining precision and consistency in their workflows.

The protocol is adaptable to both manual and automated workflows, supporting 96-well plates for high-throughput automation or single tubes for manual processing. For added flexibility, the kit offers an optional Auto-Mag® PCR-Pure reagent (available upon request), which allows users to recover any excess DNA that was not bound during the normalization process.

Features

- Rapid and Reliable Quantification & Normalization: Efficient quantification and normalization of gDNA, PCR amplicons, or fragmented DNA.
- Consistent Results: Achieves reproducible normalization results (Genomic DNA: ~400 ng, PCR Product: ~400 ng).
- Versatile Sample Processing: Suitable for pooled DNA samples from diverse sources.
- No Additional Steps: No centrifugation, filtration, or standard curve required.
- Simultaneous Actions: Normalization, clean-up, and concentration performed in a single process.
- Efficiency & Cost Reduction: Reduces library construction time, reagent consumption, and overall costs.
- Workflow Flexibility: Compatible with both manual and automated workflows.
- Unbound DNA Recovery: Includes reagents for the recovery of excess unbound DNA (Cat. # S006-01P and S006-02P).

Kit Contents

Product Number	S006-00	S006-01	S006-02	S006-01P	S006-02P
Number of Preps*	10	96	384	96	384
Auto-Mag® C-7	0.25 ml	2 ml	8 ml	2 ml	8 ml
NC Buffer	0.6 ml	6 ml	25 ml	6 ml	25 ml
Elution Buffer	1 ml	10 ml	40 ml	10 ml	40 ml
Auto-Mag® PCR-Pure	-	-	-	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, and all other components can be stored at room temperature.

During shipment or storage in cool ambient conditions, the precipitates may form in NC buffers. Check the buffer 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	gDNA, PCR amplicon, DNA fragments.
Starting Amount	Up to 50 µl
Binding capacity	Scalable
Downstream Application	NGS, qPCR,
Elution Volume	40 µl or adjustable
Processing format	Automated; Manual
Storage	Room temperatures

Auto-Mag® DNA Normalization Protocols

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
- 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 10 minutes before use.

Protocol for gDNA Normalization

The standard protocol for gDNA normalization using 20 µL of beads will recover approximately 400 ng of gDNA from a 50 µL gDNA sample.

gDNA normalization depends on the limited binding capacity of the beads. While any amount of gDNA can be used, samples with lower DNA content or variations in extraction methods may affect the beads' ability to achieve full saturation binding. For optimal normalization, the gDNA content in a 50 µL sample should be at least 1500 ng. Using less than the recommended amount of gDNA may increase variability in the normalization process by preventing full bead saturation. It is recommended that users pre-test a small portion of their sample to ensure proper normalization and recovery of the desired amount of DNA. If needed, the bead volume can be slightly adjusted to achieve the desired DNA recovery. For further assistance, please contact AMD's technical support team.

Protocol for 96-Well Plate Format (~400ng DNA Output)

1. Transfer 50µl gDNA sample 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 20µl Auto-Mag® C-7 to each sample well.

   Note: Recommendation: For use with multiple samples, Prepare master mixture of NC Buffer / Auto-Mag® C-7: (50ul NC buffer, 20ul Auto-Mag® C-7 for one sample) and add 70ul mixture to each sample well.

3. Gently mix samples by pipetting up and down 20 times and incubate at room temperature for 8 minutes.

4. 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 the plate as "DNA recovery Plate." Refer to the "Unbound DNA Recovery" protocol.

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

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

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

7. 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.

8. Remove the sample plate from the magnet. Add 40µl of Elution Buffer to each sample and gently mix by pipetting up and down 20 times.

   Note: Different volumes (15-100ul) of elution buffer can be used as required for subsequent experiments.

9. Seal the plate and incubate at 55ºC for 5 minutes.

10. Gently mix sample again by pipetting up and down 20 times or vortex for 20 seconds.

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

12. 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.

Protocol for PCR Amplicon Normalization & Cleanup

This protocol simplifies the high-throughput cleanup and normalization of PCR amplicons directly from raw PCR reaction samples. Using this standard normalization process, approximately 400 ng of PCR amplicons can be recovered from 25–50 µL PCR reaction samples using 10 µL beads.

Several factors can influence PCR amplification efficiency, including the type of PCR reagents used, Taq enzyme, template quality, primers, and the size of PCR products. The Auto-Mag® DNA Normalization Kit is specifically designed to normalize PCR products generated with a variety of PCR reagents. However, compositional differences in PCR reagents from different manufacturers, while not interfering with the normalization process, may affect the recovery of normalized PCR products. To minimize variations in normalization and recovery due to these compositional differences, it is recommended to avoid combining PCR products generated with reagents from different manufacturers in the same normalization process.

It is also recommended that users pre-test a small sample to ensure proper normalization and recovery of the desired PCR product. If necessary, the bead volume can be slightly adjusted to achieve the desired PCR recovery. For further assistance, please contact AMD's technical support team.

Protocol for 96-well Plate Format

1. Confirm the volume of PCR reaction after finishing PCR reactions. Transfer 25-50µl PCR sample to a well of 96-well plate. Label the plate as sample plate.

   Note: If the PCR reaction sample volume is less than 25µl, adjust volume to 25-50µl range with 1x PCR reaction buffer.

2. Add 50µl of NC buffer, 50µl of 100% Ethanol and 10µl of Auto-Mag® C-7 to each sample.

   Note: Recommendation: For use with multiple samples, Prepare master mixture of NC Buffer, 100% Ethanol, and Auto-Mag® C-7: (50ul NC buffer, 50ul of Ethanol, and 10ul Auto-Mag® C-7 for one sample) and add 110ul mixture to each sample well.

3. Mix samples by pipetting up and down 20 times or vortex for 20 seconds, and incubate at room temperature for 8 minutes.

4. 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 the plate as "DNA recovery Plate." Refer to the "Unbound DNA Recovery" protocol.

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

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

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

7. 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.

8. Remove the sample plate from the magnet. Add 40µl of Elution Buffer to each sample and gently mix by pipetting

up and down 20 times or vortex for 20 seconds

Note: Different volumes (15-100ul) of elution buffer can be used as required for subsequent experiments.

9. Seal the plate and incubate at 55ºC for 5 minutes.

10. Mix sample again by pipetting up and down 20 times or vortex for 20 seconds.

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

12. 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.

Unbound DNA Recovery Protocol

The following protocol is for recovery of unbound DNA from supernatant of DNA normalizing procedure

Procedure

1. Complete Steps 1-4 of the DNA Normalization protocols before beginning this procedure.

2. Confirm the volume of the supernatant and transfer the supernatant to a new 96-well plate. Label as DNA recovery plate.

3. a). For recovery gDNA, Add the same volume of Auto-Mag® PCR-Pure reagents to the sample.
   (For example: For 100µl the supernatant, add 100µl Auto-Mag® PCR-Pure reagents).

   b). For recovery PCR amplicon, Add the 1.8x volume of Auto-Mag® PCR-Pure reagents to the sample.
   (For example: For 100µl the supernatant, add 180µl 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 clear 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 and third 80% Ethanol rinse step.

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 clear 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 Yields	Less input DNA	Use at least 1,500 ng gDNA to achieve desired results. Use at least 25µL or more of the original PCR reaction products to achieve the desired results.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® DNA Normalization Kit	S006-00 S006-01 S006-02 S006-01P S006-02P S006-Bulk	10 Preps. 96 Preps. 384 Preps. 96 Preps 384 Preps Request.

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 250 ml 500 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

Trademarks