AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® PCR-Pure

Version 2.1

Magnetic beads-based reagent for automated or manual PCR, DNA, purification, and DNA fragment size selection

Catalog Number: S002-01, S002-02, S002-03, S002-04

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

Auto-Mag® PCR-Pure consists of AMD's own paramagnetic beads and optimized chemicals that is specifically designed for efficient purification of amplicons and tight size-specific selection of DNA fragments. Purification consists of removal of salts, primers, primer-dimers, dNTPs, as DNA fragments are selectively bound to the magnetic bead particles. Highly purified DNA is eluted with low salt elution buffer or water which can be used directly for downstream applications, such as library construction, next generation sequencing (NGS), Sanger sequencing, cloning, restriction digestions, adapter ligation, microarrays, and so on. Auto-Mag® PCR-Pure is suitable for manual procedures as well as in automation.

Features:

- No protocol changes against major competitor
- Rapid and reliable post-PCR and post enzymatic reaction clean-up
- High recovery of amplicons or dsDNA fragments greater than 100bp
- Sample: up to 100µl of PCR products
- Uniform fragments size distribution
- 96- or 384-well formats
- Adaptable to high throughput liquid handling workstations
- Significant cost savings

Kit Contents

Product Number	S002-01	S002-02	S002-03	S002-04
Auto-Mag® PCR-Pure	5 ml	50 ml	250 ml	500 ml

Storage and Stability

Auto-Mag® PCR-Pure is shipped at room temperature and is stable for at least 12 months from the date of purchase when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Preparation of Reagents

1. Prepare 80% Ethanol for DNA Wash. (Prepare from absolute ethanol. Do not use denatured alcohol).

   Fresh prepare 80% ethanol, keep cover tight and use in one week.

2. Molecular biology grade water, Tris (10 mM, pH 8.0) or TE (10 mM Tris, pH 8.1 mM EDTA) for DNA elution

Additional Information

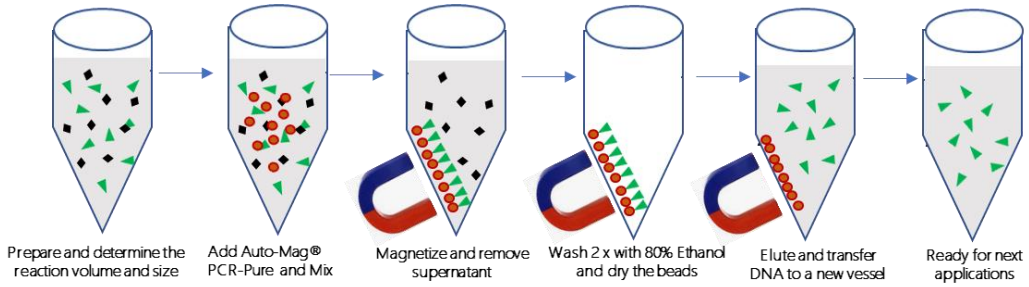
1. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	DNA or RNA, PCR amplicon, double stranded DNA fragment, gDNA
Starting amount	Scalable
DNA recovered	>90% recovery for DNA >100bp
Downstream Application	NGS, PCR, qPCR, Cloning, Nucleic Acid Labeling, Mutation detection, genotyping, Southern Blotting etc.
Elution Volume	15 µl or above
Processing format	Automated; Manual
Storage	2°C - 8°C

2. Performing manually without access to a magnet

The compatible magnetic separation device is required to pellet the magnetic particles When performing Auto-Mag® PCR-Pure protocol. If performing the protocol manually without access to a magnet, sample tubes or plates can be centrifuged for 30 seconds (single tubes: full speed; plates: 3,000 x g) to enable the magnetic particles to form a pellet. All processes are to be carried out at room temperature (15–25 °C).

3. PCR Amplicon Purification Process Overview



1. Confirm the volume of PCR reaction samples.
2. Add 1.8 µl Auto-Mag® PCR-Pure per 1 µl of sample.
3. Separation of beads/DNA fragment from contamination.
4. Wash beads/DNA twice with 80% Ethanol to remove contaminants.
5. Elution purified DNA fragment from beads.
6. Transfer eluate to new vessel and ready for next applications

The detailed procedure for PCR purification can be found in the following the 96 Well Format, Single-tube format, or 384 well format purification procedure in this manual.

Auto-Mag® PCR-Pure Protocols for DNA/PCR clean-up

Auto-Mag® PCR-Pure offers a fast and convenient way to cleanup PCR products, DNA, or other enzymatic reactions with high recovery rates. Auto-Mag® PCR-Pure typically uses the 1:1.8x volumetric ratio of samples. Amplicons or DNA fragments >100bp are retained while smaller fragments, primers, linkers, enzymes, and other buffer components are effectively removed. Highly purified PCR product or DNA fragments is then eluted with low salt elution buffer or water which can be used directly for downstream applications,

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.5-2.0 ml tube, and Magnetic Rack Separator for tube. (Any vendor of choice).
- 96-well format: 96 well thermal cycling plate, or 300µl round bottom microtiter plate, or 1.2 ml deep well microtiter plate and appropriate magnetic separation device (www.fishersci.com or any vender of choice).
- 384-well format: 384 well (40µl well capacity) cycling plate and appropriate magnetic separation device (www.fishersci.com or any vender of choice).
- Laboratory mixer, vortex, or equivalent.
- 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- RNase/DNase free Elution buffe (AMD-B232 www.amdbiotech.com), or Either water, TRIS-Acetate (10 mM pH 8.0), or TE Buffer (10 mM Tris-Acetate pH 8.0, 1 mM EDTA) for DNA elution.
- Well calibrated pipettor and Disposable pipette tips.

Before Starting

- Prepare 80% Ethanol for DNA wash steps according to the instructions of Preparation Reagents on page 3.
- Bring the Auto-Mag® PCR-Pure reagent bottle to room temperature for at least 30 minutes before use.
- Shake thoroughly the Auto-Mag® PCR-Pure reagent to fully resuspend the magnetic beads.

Protocol for PCR Amplicon Purification: 96 Well Format, or Single-Tube Format

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.

2. Confirm the volume of PCR reaction and determine whether a plate transfer is necessary for 96 well plate, or transfer the sample to a new 1.5-2.0 ml tube.

   Note: PCR plates generally have a maximum volume of 200 µl. If the reaction volume multiplied by 2.8 exceeds the volume of the PCR plate, transfer PCR reaction to a 300ul round plate or a 1.2 ml deep-well plate.

3. Reference Table below, add the appropriate volume of Auto-Mag® PCR-Pure reagent to the PCR sample.

PCR Reaction Volume (µl)	Auto-Mag® PCR-Pure Volume at 1.8x (µl) *
10	18
20	36
25	45
50	90
100	180
(Volume of Auto-Mag® PCR-Pure reagent per reaction) = 1.8 X (PCR reaction volume).	

4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette 10 times or vortexing for 10 seconds.

   Note: This step binds DNA fragments 100 bp and larger to the magnetic beads.
   If a 96 well plate and vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Incubate samples at room temperature for 5 minutes for maximum recovery.

6. Place the sample tubes or plate on a compatible magnetic separation device for 2 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample still on the magnet, remove and discard the supernatant by pipetting. Do not disturb the attracted beads.

8. With the sample on the magnet, add 200µl of 80% ethanol to each sample and incubate at room temperature for 30 seconds. Mixing is not necessary.

9. With the sample still on the magnet, remove and discard the supernatant by pipetting.

   Note: If the total volume of sample plus reagent exceeds 200µl, then use a wash volume of at least the volume of sample plus reagent.

10. Repeat steps 8-9 for a second 80% ethanol wash.

11. Dry the beads by incubating the plate at room temperature for 5 minutes with the samples still on the magnetic separation device. 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 significantly decrease elution efficiency.

12. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) to each sample and mix by pipet up and down 10 times.

    Note: Prewarming the elution buff er to 55°C can increase the yield.

13. Incubate the sample at room temperature for 2 minutes.

14. Place the sample back on the magnet and wait 2 minutes or until the magnetic beads are completely cleared from solution.

15. Transfer the eluate to an appropriate storage vessel and keep at -20°C for long term storage or for subsequent applications.

Protocol for PCR Amplicon Purification: 384 Well Format

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.

2. Confirm the volume of PCR reaction and determine volume of Auto-Mag® PCR-Pure reagent needed.

3. Reference Table below, add the appropriate volume of Auto-Mag® PCR-Pure reagent to the PCR sample

   Note: Generally, 384-well thermal cycling plates have a maximum well volume of 40µl. Reactions of 14µl or less can be purified in this type of plate (14µl x 2.8 = 39.2µl).

PCR Sample Reaction Volume (µl)	Auto-Mag® PCR-Pure Volume at 1.8x (µl) *
5	9
7	12.6
10	18
14	25.2
(Volume of Auto-Mag® PCR-Pure reagent per reaction) = 1.8 X (PCR reaction volume).	

4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette 10 times or vortexing for 10 seconds.

   Note: This step binds DNA fragments 100 bp and larger to the magnetic beads. If vortex is used for mixing, the plate must be sealed with a plate seal before vortexing.

5. Incubate samples at room temperature for 5 minutes for maximum recovery.

6. Place the reaction plate on a 384 magnetic separation device for 2 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample plate still on the magnet, remove and discard the supernatant by pipetting. Do not disturb the attracted beads.

8. With the plate on the magnet, add 30µl of 80% ethanol to each sample and incubate at room temperature for 30 seconds. Mixing is not necessary.

9. With the plate still on the magnet, remove and discard the supernatant by pipetting.

10. Repeat steps 8-9 for a second 80% ethanol wash.

11. Dry the beads by incubating the plate at room temperature for 5 minutes with the plate still on the magnetic separation device. 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 significantly decrease elution efficiency.

12. Remove the sample plate or tubes from the magnet. Add 20µl of elution buffer (reagent grade water, or TE buffer) to each sample and pipet up and down 5 times to mix.

    Note: Prewarming the elution buff er to 55°C can increase the yield.

13. Incubate the sample at room temperature for 2 minutes.

14. Place the plate back on the magnet and wait 2 minutes or until the magnetic beads are completely cleared from solution.

15. Transfer the eluate to an appropriate storage vessel and keep at -20°C for long term storage or for subsequent applications.

Protocol for gDNA Clean-up: 96 Well Format, or Single-Tube Format

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.

2. Confirm the volume of PCR reaction and determine whether a plate transfer is necessary for 96 well plate, or transfer the sample to a new 1.5-2.0 ml tube.

3. Reference Table below, add the appropriate volume of Auto-Mag® PCR-Pure reagent to the sample.

gDNA Sample Volume (µl)	Auto-Mag® PCR-Pure Volume at 0.8x (µl) *
10	8
20	16
30	24
40	32
50	40
100	80
(Volume of Auto-Mag® PCR-Pure reagent per reaction) = 0.8X (gDNA sample volume).	

4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette 10 times or vortexing 10 seconds.

   Note: If a 96 well plate and vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Incubate samples at room temperature for 5 minutes for maximum recovery.

6. Place the sample tubes or plate on a compatible magnetic separation device for 2 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample still on the magnet, remove and discard the supernatant by pipetting. Do not disturb the attracted beads.

8. With the sample on the magnet, add 200µl of 80% ethanol to each sample and incubate at room temperature for 30 seconds. Mixing is not necessary.

9. With the sample still on the magnet, remove and discard the supernatant by pipetting.

   Note: If the total volume of sample plus reagent exceeds 200µl, then use a wash volume of at least the volume of sample plus reagent.

10. Repeat steps 8-9 for a second 80% ethanol wash.

11. Dry the beads by incubating the plate at room temperature for 5 minutes with the plate still on the magnetic separation device. 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 significantly decrease elution efficiency.

12. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) to each sample and pipet up and down 5 times to mix.

    Note: Prewarming the elution buff er to 55°C can increase the yield.

13. Incubate the sample at room temperature for 2 minutes.

14. Place the sample back on the magnet and wait 2 minutes or until the magnetic beads are completely cleared from solution.

15. Transfer the eluate to an appropriate storage vessel and keep at -20°C for long term storage or for subsequent applications.

Customized Protocol and Programmed Procedure for Automation Purification

To obtain a custom protocol for DNA fragment size selection of a specific fragment size; or automating this procedure on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions or additional processing procedures.

Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

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 yield/ Incorrect recovery of PCR purification	Recovery was measured by Spectrophotometry Absorbance. This may cause the recovery to appear lower than it is.	Run sample on an agarose gel to double check the recovery measurement or use a quantitative double-stranded DNA assay reagent such as PicoGreen assay.
	Bead Loss	If beads get aspirated into tips during supernatant removal, the nucleic acid bound to these beads will also be lost. Aspirate slowly and remove as much of the first supernatant as possible without disturbing the bead.
	Insufficient Mixing	Mixing thoroughly during the initial bind mix and elution mix is critical. to ensure the beads get sufficiently resuspended.
	Large Reaction Volume	Large volume reactions can benefit from an extended binding and separation time. Increase binding time to 10 minutes and ensure all beads are separated before removing the supernatant.
	Low Elution Volume	A small elution volume leads to a decrease in recovery. This is because a small amount of elution buffer always stays behind coating the beads. To increase the elution volume.
Fragment size incompatible	Insufficient ratio	Use volume ratios outlined in this manual.
	Insufficient ethanol concentration used for washing step	Use freshly prepared 80% ethanol. Over time ethanol becomes more diluted through evaporation and absorption of atmospheric water. Therefore, parts of the DNA pellet go into solution and DNA fragments are washed away.
	Elution buffer volume insufficient	Bead pellet must be covered completely with elution buffer
	Beads over dried	Do not dry beads for longer than 15 minutes at room temperature. Over drying of beads may result in lower elution efficiencies.
Downstream applications are unsuccessful	Carry-over of ethanol from washing step	Be sure to remove all the ethanol after the final wash step. Dry beads 5-10 minutes at room temperature.
Carry-over of beads	Time for magnetic separation too short	Increase separation time to allow the beads to be attracted to the magnetic pins completely

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® PCR-Pure	S002-01 S002-02 S002-03 S002-04	5 ml 50 ml 250 ml 500 ml
Auto-Mag® DNA Elution Buffer	B228-01 B228-02 B228-03	50 ml 250 ml 500 ml

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