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Advanced Molecular Detection

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Auto-Mag® PCR-Pure

Cat # AMD-S002 Version 2.0

- Magnetic beads-based reagent for manual and automated post-PCR and post-enzymatic reaction clean-up, NGS library clean-up, and reliable amplicon size selection.

Highlights

- Designed for "bottle swap" with no protocol change against major competitor
- One reagent for DNA, or cDNA purification, PCR products post-cleanup and DNA fragment size selection
- Efficiently removes unincorporated dNTPs, primers, primer dimers and other contaminants.
- High recovery of amplicons or dsDNA fragments greater than 100bp
- Double-sided size selection capability and uniform fragments size distribution
- No centrifugation or filtration needed. Manual and automation-friendly sample processing in 96- and 384-well formats

Protocol Contents

Disclaimers and Safety Information

This kit is designed for research purposes only. The all biological samples are considered potentially infectious. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

Trademarks

The trademarks mentioned herein are the property of AMD Biotech Inc. or their respective owners.

Product Introduction

Auto-Mag® PCR-Pure is designed for quick high-throughput purification of DNA fragments from PCR or NGS workflows with high recovery rates. Auto-Mag® PCR-Pure consists of AMD's paramagnetic particles and optimized chemicals that selectively recovers amplicons greater than 100 bp with consistency and reliability. It is also capable of selectively binding fragments depending on the reagent-to-sample ratio used, giving the user flexibility to perform left, right, or double-sided size selection. This product can be used for both manual and fully automated purification of DNA samples. Purified DNA is suitable for a variety of downstream applications such as NGS library preparation, microarrays, automated fluorescent sequencing as well as for post PCR clean-up of shorter amplicons or restriction enzyme digestion products.

Applications

DNA fragments purified with Auto-Mag® PCR-Pure are ready to be used in the following applications:

- PCR
- NGS library preparation
- Amplicon size selection for NGS
- Microarrays
- Sanger sequencing
- Restriction enzyme digestions, adapter ligations
- Cloning

Kit Contents

Product Number	AMD-S002-5	AMD-S002-50	AMD-S002-250	AMD-S002-500
Auto-Mag® PCR-Pure	5 ml	50 ml	250 ml	500 ml
Number of Preparation*	278	2,778	13,890	27,780

*The number of preps is based on typical 10µl PCR reaction volume, and the ratio volume of Auto-Mag® PCR-Pure reagent per reaction = 1.8 x (PCR Reaction Volume)

Storage and Stability

Auto-Mag® PCR Pure is shipped at room temperature and is guaranteed for at least 12 months from the date of purchase when the kit is stored at 2-8°C after received. Do not freeze at any time.

Basic Protocol for PCR /DNA clean-up

Materials and Equipment to be supplied by User

- 100 % Ethanol.
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer).
- For 96 well format: 96 well cycling plate
- ~1.5 ml Nuclease free tubes.
- Appropriate magnetic separation device compatible with 96-well PCR plate or 1.5 ml tubes.
- Well calibrated pipettor and disposable pipette tips.

Before Starting

- Prepare fresh 80% Ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

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

2. Add the appropriate volume of Auto-Mag® PCR-Pure reagent into the reaction vessels containing 10-100µl PCR/DNA sample according to the instructions in the table below.

Table 1: Some common sample volumes and suggested Auto-Mag® PCR-Pure volumes

Sample Volume (µl)	Auto-Mag® PCR-Pure Volume (µl)*
10	18
20	36
25	45
50	90
100	180

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for reaction: Auto-Mag® PCR-Pure reagent volume per reaction = 1.8 X sample volume.*

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

4. Place the sample reaction vessels on the 96-well, or appropriate magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

5. Keep the sample reaction vessels on the magnet and add 200µl of 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second 80% ethanol wash.

7. Keep the sample reaction vessels on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

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

8. Remove the sample reaction vessels from the magnetic separation device. Add 20-50µl of elution buffer (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each well and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

9. Place the sample reaction vessels back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.

Protocol for PCR clean-up (96-well Plate Format)

The following protocol exemplifies for removal of salts, primers, primer-dimers, dNTPs, form PCR reactions and selectively recovers amplicons greater than 100 bp.

Materials and Equipment to be supplied by User

- 100 % ethanol.
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer).
- For 96 well format: 96 well cycling plate.
- Appropriate magnetic separation device compatible with 96-well PCR plate.
- Well calibrated pipettor and Disposable pipette tips.

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

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

2. Add the appropriate volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-50ul PCR sample according to the instructions in the table below.

Table 2: Some common PCR reaction volumes and suggested Auto-Mag® PCR-Pure volumes

PCR Sample Volume (ul)	Auto-Mag® PCR-Pure Volume (ul)*
10	18
20	36
25	45
50	90

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for PCR reaction: Auto-Mag® PCR-Pure reagent volume per reaction = 1.8 X PCR reaction volume.*

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

4. Place the sample plate on the 96 well magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second 80% ethanol wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

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 20-50µl of elution buffer (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each well and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to a new plate and keep at -20°C for long term storage, or for subsequent applications.

Protocol for DNA clean-up and single size selection (96-well Plate Format)

This protocol can be used to remove contaminants (such as, nucleotides, primers, adapters, enzymes, buffer additives, salts) and shorter DNA fragments, less than150–200 bp from a sample.

Materials and Equipment to be supplied by User:

- 100 % ethanol
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer)
- For 96 well format: 96 well cycling plate
- Appropriate magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

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

2. Add 1.0 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100ul DNA sample according to the instructions in the table below.

Table 3: Some common reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

DNA Sample Volume (ul)	Auto-Mag® PCR-Pure Volume (ul)*
10	10
20	20
25	25
50	50
100	100

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for DNA sample: Auto-Mag® PCR-Pure reagent volume per reaction = 1.0 X DNA sample volume.*

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

4. Place the sample plate on the 96 well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second 80% ethanol wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

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 20-50µl of elution buffer (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each well and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to a new plate and keep at -20°C for long term storage, or for subsequent applications.

Protocol for removing adapter dimers (96-well Plate Format)

This protocol can be used to remove un-ligated adapters and adapter dimers after an adapter addition reaction in NGS library construction.

Materials and Equipment to be supplied by User:

- 100 % ethanol
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer)
- For 96 well format: 96 well cycling plate
- Appropriate magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

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

2. Add 0.8 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100ul adapter addition reaction sample according to the instructions in the table below.

Table 4: Some common adapter addition reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

Adapter addition reaction Sample Volume (ul)	Auto-Mag® PCR-Pure Volume (ul)*
25	20
50	40
65	52
100	80

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for the sample: Auto-Mag® PCR-Pure reagent volume per reaction = 0.8 X sample volume.*

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

4. Place the sample plate on the 96 well magnetic separation device and allow Auto-Mag® RNA-Pure beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second 80% ethanol wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

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 20-50µl of elution buffer (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each well and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

Note: Pre-warming the elution buffer at 55°C can increase the yield.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.

Note: If necessary, perform a second 0.8x bead-based clean-up.

Additional clean-up

11. Add 0.8 volume of Auto-Mag® PCR-Pure reagent to the supernatant collected from step 10.

12 Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting up and down 10 times. Incubate the mixture for 5 minutes at room temperature.

13. Repeat steps 4-7 (capture and washes)

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of RNase/DNase free elution buffer, (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each sample and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.

Protocol for DNA double-sided size selection (96-well Plate Format)

This protocol can be used to generate DNA fragment libraries with a certain size range or to narrow fragment-size distribution. Through a two times selections process, the both smaller and larger fragments can be removed and the expected size range of DNA fragments can be obtained. This method is called double-sided size selection. The following protocol exemplifies a size selection of DNA fragment libraries with a size range of 250–400bp from a 50ul DNA samples. The information provided is an approximate guide. By altering the volume ratios of DNA fragment libraries and PCR-Pure reagents, other size ranges can be obtained. The optimal ratio for other size ranges should be determined empirically

Materials and Equipment to be supplied by User:

- 100 % Ethanol
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer)
- For 96 well format: 96 well cycling plate
- Appropriate magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

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

2. For using the 96-well plate, a starting sample volume of 50µl is recommended. For smaller samples, add nuclease-free water to adjust the volume.

3. For removing the large DNA fragments, first add 35µl of well dispensed Auto-Mag® PCR-Pure reagent into the wells containing 50µl DNA sample. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The volume ratio of Auto-Mag® PCR-Pure reagent suspension to the sample is 0.7x.

4. Incubate the mixture for 5 minutes at room temperature to allow the large DNA fragments are bound to Auto-Mag® PCR-Pure beads.

5. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear.

6. Keep the sample plate on the magnet and transfer the ~85µl of supernatant into the well of a new plate and discard the beads that contain the unwanted large DNA fragments.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

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

8. For the smaller DNA fragments cutoff, add 15µl of well dispersed Auto-Mag® PCR-Pure reagent into the well containing supernatants from step 6. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The total volume ratio of Auto-Mag® PCR-Pure reagent suspension to the original sample is 1.0 x now; (35µl and 15µl to 50µl).

9. Incubate the sample plate for 10 minutes at room temperature to allow the selected DNA fragment are bound to Auto-Mag® PCR-Pure beads.

10. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

11. Keep the sample plate on the magnet and add 200µl of 80 % ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: DNA Wash Buffer must be diluted with ethanol prior to use.

12. Repeat Steps 11 for second 80% ethanol wash.

13. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

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

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer all of supernatant to a new 96-well plate or an appropriate storage vessel. The ultra-pure DNA fragments (250-400bp) are ready for next step of NGS library preparation process or store at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 1-404-259-6276 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low DNA yield	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 dilute 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 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