AMD Biotech Inc.
Advanced Molecular Detection
Product Manual

Auto-Mag® PCR-Pure

Version 1.0

Magnetic beads-based chemistry for PCR, DNA, purification, and DNA fragment size selection

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

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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 selection of sheared DNA fragments and amplicon purification. Fragment size selection by Auto-Mag® PCR-Pure is always tight and consistent. The purification process consists of removal of salts, primers, primer-dimers, dNTPs, salts, enzymes, adapters, and adapter dimers DNA amplicons and fragments are selectively bound to the magnetic beads' particles; and highly purified DNA is eluted with low salt elution buffer or water which can be used directly for downstream applications. Auto-Mag® PCR-Pure is compatible with most next generation sequencing platforms.

Intended use:

Auto-Mag® PCR-Pure uses a magnetic bead-based chemistry for cleaning up DNA amplicons and binding of target DNA fragment of the desired size range. The kit is intended for robust and efficient amplicon clean-up and DNA fragment selection in next generation sequencing library preparation. Auto-Mag® PCR-Pure purified products can be used in the following applications:

- PCR
- Sequencing
- Genotyping
- Fragment Analysis
- Primer Walking
- Cloning. etc.

Product Number	S002-01	S002-02	S002-03	S002-04
Auto-Mag® PCR-Pure	5 ml	50 ml	500 ml	1000 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).

Ethanol is hygroscopic. When opened the ethanol will both evaporate and absorb water over time. Fresh prepare 80% ethanol then keep cover tight and use in one week.

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).

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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,

Auto-Mag® PCR-Pure can be used for PCR purification in single-tube, 96 well and 384 well format. The following tables illustrate the number of PCR reactions the Auto-Mag® PCR-Pure will purify depending on the format required by the user.

Table 1. Number of PCR Reactions Purified with 96 Well Format.

PCR Reaction Volumes 96 Well Format (µl)	Product Number S002-01	Product Number S002-02	Product Number S002-03	Product Number S002-04
10	278 rxns	2,778 rxns	27,778 rxns	55,555 rxns
20	139 rxns	1,389 rxns	13,889 rxns	27,778 rxns
25	111 rxns	1,111 rxns	11,111 rxns	22,222 rxns
50	56 rxns	556 rxns	5,556, rxns	11,111 rxns
100	28 rxns	278 rxns	2,778 rxns	5,555 rxns

Table 2. Number of PCR Reactions Purified with 384 Well Format.

PCR Reaction Volumes 384 Well Format (µl)	Product Number S003-01	Product Number S003-02	Product Number S003-03	Product Number S003-04
5	556 rxns	5,556 rxns	55,5556 rxns	111,111 rxns
7	397 rxns	3,968 rxns	39,683 rxns	79,365 rxns
10	278 rxns	2,778 rxns	27,778 rxns	55,555 rxns
14	198 rxns	1,984 rxns	19,841 rxns	39,683 rxns

Materials and Equipment to Be Supplied by User

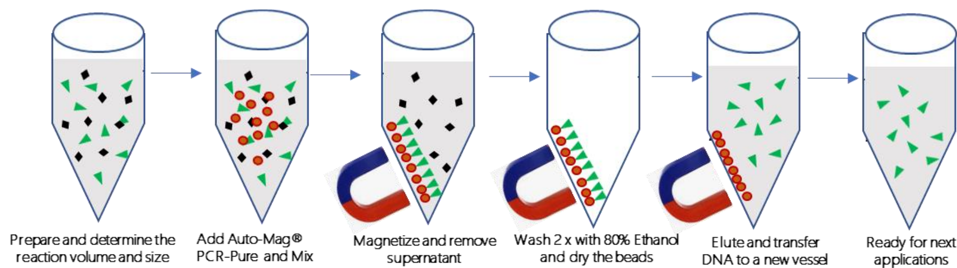
- Single-tube format: Nuclease-free 1.5-2.0 ml tube, and Magnetic Rack Separator for tube. (Any vendor of choice).
- 96-well format: 96 well thermal cycling plate, or 300µl round bottom microtiter plate, or 1.2 ml deep well microtiter plate and appropriate magnetic separation device (www.fishersci.com 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).
- Plate Seals, Adhesive or Heat (AB-3739; www.fishersci.com or any vender of choice).
- Automated platforms for magnetic bead purification (Automated DNA recovery).
- 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.
- Suspension Auto-Mag® PCR-Pure by vortexing.

PCR Amplicon Purification Process Overview



The workflow for the PCR purification process is as follows:

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.

Procedure 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. Add the appropriate volume of Auto-Mag® PCR-Pure reagent to the PCR sample reaction volume shown in Table 3.

Table 3: Auto-Mag® PCR-Pure to Sample Reaction Volume Chart

PCR Sample Reaction Volume (µl)	Auto-Mag® PCR-Pure Volume Needed 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 mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes for maximum recovery.

   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. Place the reaction plate or tubes on a compatible magnetic separation device for 2-5 minutes or until the magnetic beads are completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

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

   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.

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

8. Keep the sample 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 significantly decrease elution efficiency.

9. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) and pipette mixing 20 times or vortexing for 20 seconds. Ensure beads are no longer attached to the side of the well.

   Note: To ensure the elution buffer encounters the beads, if necessary, a greater volume of elution buffer can be used.
   If a 96 well plate and vortexing is used, the plate must be sealed with a plate seal before vortexing.

10. Incubate the sample at room temperature for 5 minutes.

11. Place the sample plate or tubes back on the magnet and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

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

Procedure for Removing Adapter Dimers: 96 Well Format, or Single-Tube Format

The adapter dimer is a byproduct formed in NGS library preparation. If not removed, the adapter dimers will compete for binding to the flow cell and for PCR amplification of the desired target ligated to adapters, and significantly lower the sequencing efficiency and quality. Auto-Mag® PCR-Pure offers a fast and convenient way to remove the adapter dimers.

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

2. Conform the volume of adapter addition reaction sample, Transfer sample into a tube or well of 96-well plate.

3. Add the appropriate volume of Auto-Mag® PCR-Pure reagent to the sample reaction volume shown in Table 4.

Table 4: Auto-Mag® PCR-Pure to Sample Reaction Volume Chart

Adapter addition reaction Sample Volume (µl)	Auto-Mag® PCR-Pure Volume Needed (µl) *
20	18
25	2.5
35	31.5
50	45
65	58.5
(Volume of Auto-Mag® PCR-Pure reagent per reaction) = 0.9 X (sample volume).	

4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes for maximum recovery.

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

5. Place the reaction plate or tubes on a compatible magnetic separation device for 2-5 minutes or until the magnetic beads are completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

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

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

8. Keep the sample 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 significantly decrease elution efficiency.

9. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) and pipette mixing 20 times or vortexing for 20 seconds. Ensure beads are no longer attached to the side of the well.

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

10. Incubate the sample at room temperature for 5 minutes.

11. Place the sample plate or tubes back on the magnet and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

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

Procedure 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. Conform the volume of gDNA sample and transfer sample into a tube or well of 96-well plate.

3. Add the appropriate volume of Auto-Mag® PCR-Pure reagent to the sample reaction volume shown in Table 5.

Table 5: Auto-Mag® PCR-Pure to Sample Reaction Volume Chart

gDNA Sample Volume (µl)	Auto-Mag® PCR-Pure Volume Needed (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 mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes for maximum recovery.

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

5. Place the reaction plate or tubes on a compatible magnetic separation device for 2-5 minutes or until the magnetic beads are completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

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

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

8. Keep the sample 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 significantly decrease elution efficiency.

9. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) and pipette mixing 20 times or vortexing for 20 seconds. Ensure beads are no longer attached to the side of the well.

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

10. Incubate the sample at room temperature for 5 minutes.

11. Place the sample plate or tubes back on the magnet and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

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

Auto-Mag® PCR-Pure: Protocols for DNA Size Selection & cleanup

Auto-Mag® PCR-Pure can selectively bind DNA fragments based on the ratio of Auto-Mag® PCR-Pure reagent to sample. Adjusting the ratio will control to eliminate smaller or larger fragment sizes not within the target range and the desired size DNA Fragment are selected and recovered.

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).
- Plate Seals, Adhesive or Heat (AB-3739; www.fishersci.com or any vender of choice).
- Automated platforms for magnetic bead purification (Automated DNA recovery).
- 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.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Sample preparation

- DNA samples should be fragmented double-stranded DNA and dissolved in molecular biology grade water or standard buffer solution such as Tris or TE.
- For best results, the sample volume should be ≥ 50µl. A lower volume will decrease pipetting accuracy, therefore increasing selection point variability.

- For a Left Side-Size Selection, the majority of DNA fragment size distribution should be larger than the selected cutoff point.
- for a Right-Side Size Selection, the majority of DNA fragment size distribution should be smaller than selected cutoff point.
- For a Double Size Selection, most of size distribution should be centered between the selection points and in general, the range of DNA fragments may be no smaller than 100 bp and no larger than 800 bp.

Procedure for DNA Double Size Selection: 96 Well Format, or Single-Tube Format

The DNA Double Size Selection method can be used to generate DNA fragment libraries with a certain size range or to narrow fragment-size distribution in the NGS library preparation,

To perform Double Size Selection, the upper cut-off is performed first, with fragments that are too large being bound to the beads. The supernatant is retained, containing all fragments below the specified upper cut-off size, and is used as starting solution for the second lower cut-off. The subsequent lower cut-off binds all fragments greater than the specified size to the beads whilst smaller fragments remain in the supernatant and are discarded. At this point, fragments within the desired range are bound to the beads and the standard washing and elution steps can be performed. To adjust the size range and center point of the final population, refer to Table 6.

Table 6: Reference conditions for DNA Double Size Selective

The average size of selection (bp)	150-220	200-300	250-320	280-350	300-400	400-550	500-700
1st ratio of Auto-Mag® PCR-Pure / DNA	1.0x	0.9x	0.8x	0.7x	0.6x	0.55x	0.5x
2nd ratio of Auto-Mag® PCR-Pure / DNA	0.2x	0.2x	0.2x	0.2x	0.2x	0.15x	0.15x
Total ratio	1.2x	1.1x	1.0x	0.9x	0.8x	0.7x	0.65x

Following is an example procedure for a 50µl input sample and a population of dsDNA fragments with an average size at 250-320bp.

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

2. Transfer 50µl sheared DNA sample into a tube or well of 96-well plate.

3. Reference Table 8, first add 40µl of Auto-Mag® PCR-Pure reagent to the sample for removing unwanted larger DNA fragments.

   Note: The volume of Auto-Mag® PCR-Pure to add = volume of sample x 2nd ratio chosen. (40µl =50 µl x 0.8x)

4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes for maximum recovery.

   Note: This step binds DNA of the upper cut-off to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. If a 96 well plate and vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 minutes, or until the Auto-Mag® PCR-Pure bead is completely cleared from solution.

6. Keep the sample on the magnet, transfer ~90µl supernatant into new tube or the well of a new plate. Discard the beads that contain the unwanted large DNA fragments.

Note: Do not disturb the attracted magnetic beads while transferring the supernatant. Significant bead transfer will cause tailing into the larger size range.

7. Reference Table 8, Add an additional 10µl of well dispersed Auto-Mag® PCR-Pure reagent into the supernatant from step 6.

Note: The volume of Auto-Mag® PCR-Pure to add = volume of sample x 2nd ratio chosen. (10µl =50 µl x 0.2x) The total ratio of Auto-Mag® PCR-Pure reagent suspension to the original sample is 1.0 x now. ((40µl + 10µl) / 50µl=1.0x).

8. Mix well by pipetting 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes.

9. Place the sample tubes or plate on the magnet for 3-5 minutes, or until the Auto-Mag® PCR-Pure bead is completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

10. With the samples still 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: 80% ethanol must be freshly made.

11. Repeat steps 10 for second 80% ethanol wash.

12. Keep the sample 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.

13. Remove the sample plate or tubes from the magnet. Add 20-50µl of elution buffer (reagent grade water, or TE buffer) and mix by pipetting up and down 20 times. Ensure beads are no longer attached to the side of the well.

Note: Depending on the downstream application, you can add any volume of Elution Buffer to elute the DNA. However, elution volumes <25% of the starting sample volume can be difficult to work with and may result in some yield loss due to the resin void volume.

14. Incubate the sample at room temperature for 5 minutes.

15. Place the sample tubes or plate back on the magnet and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

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

Procedure for NGS Library Size Selection: 96 Well Format, or Single-Tube Format

The Auto-Mag® PCR-Pure Reagent can be used for single-sided or double-sided size selection during NGS library prep. By varying the beads ratio, DNA fragments of different sizes are bound to the beads.

Single-sided size selection for NGS library prep follows essentially the same procedure as above (Procedure for PCR amplicon Purification), except that the beads ratio is adjusted to capture the different desired library DNA length. A general guideline is provided below for 50µl NGS library sample but can be fine-tuned as needed. refer to Table 7.

Table 7. recommended ratio of captured library DNA length to Auto-Mag® PCR-Pure

NGS Library Fragments to capture	Recommended Ratio	Auto-Mag® PCR-Pure Needed (µl) (For 50µl sample)
≥ 450 bp	0.6x	30 µl
≥ 300 bp	0.8x	40 µl
≥ 250 bp	0.9x	45 µl
≥ 150 bp	1.5x	75 µl
≥ 100 bp	1.8x	90 µl

Double-sided size selection for NGS library prep removes both larger and smaller DNA fragments making it ideal for preparing libraries optimized for the sequencing chemistry of choice. The following procedure is a 0.7x-0.2x selection, which generates 280-350bp NGS library fragments. Other ratios can be used to fine-tune the selection range.

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.
2. Transfer 50µl NGS library prep sample into a tube or well of 96-well plate.
3. Add 35µl of Auto-Mag® PCR-Pure reagent to the sample for removing unwanted larger DNA fragments.
4. Mix the Auto-Mag® PCR-Pure reagent and the samples thoroughly by pipette mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes for maximum recovery.
5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 minutes, or until the Auto-Mag® PCR-Pure bead is completely cleared from solution.
6. Keep the sample on the magnet, transfer 80µl supernatant into new tube or the well of a new plate. Discard the beads that contain the unwanted large DNA fragments.

   Note: Do not disturb the attracted magnetic beads while transferring the supernatant. Significant bead transfer will cause tailing into the larger size range.

7. Add an additional 10µl of well dispersed Auto-Mag® PCR-Pure reagent into 80µl supernatant samples.
8. Mix well by pipetting 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes.
9. Place the sample tubes or plate on the magnet for 3-5 minutes, or until the Auto-Mag® PCR-Pure bead is

completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

10. With the samples still 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: 80% ethanol must be freshly made.

11. Repeat steps 10 for second 80% ethanol wash.

12. Keep the sample 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.

13. Remove the sample tubes or plate from the magnet. Add 55µl of elution buffer and mix by pipetting up and down 20 times. Ensure beads are no longer attached to the side of the well.

14. Incubate the sample at room temperature for 5 minutes.

15. Place the sample tubes or plate back on the magnet and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

16. Transfer the 50 µl eluate to an appropriate storage vessel for subsequent applications.

To obtain a custom protocol for DNA size selection of a specific fragment size, please contact: 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 causes 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 500 ml 1000 ml
Auto-Mag® DNA Elution Buffer	B228-01 B228-02 B228-03	50 ml 250 ml 500 ml

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