Auto-Mag® PCR-Pure

Version 1.0

Magnetic beads-based chemistry for DNA clean up and fragment size selection of NGS library construction

S002-01	5 ml
S002-02	50 ml
S002-03	500 ml
S002-04	1000 ml

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Disclaimers and Safety Information

This kit is designed for research use only. The all-biological samples are considered potentially infectious. For more information, please consult the appropriate Material Safety Data Sheets. Information in this document is subject to change without notice.

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Auto-Mag® PCR-Pure

Product Introduction

Auto-Mag® PCR-Pure consists of AMD's own paramagnetic beads and optimized chemicals that is designed for high-throughput clean up PCR and DNA fragments, or the size selection of DNA fragments in the library construction process for next generation sequencing (NGS) with high recovery rates. In the size selection process, Auto-Mag® PCR-Pure can selectively bind fragments based on the ratio of Auto-Mag® PCR-Pure reagent to sample. Altering the ratio gives the user the ability to selectively keep or discard undesired fragment sizes. Auto-Mag® PCR-Pure is suitable for both manual and fully automated processing and uses a simple 3 steps procedure: Bind-Wash-Elute. Purified DNA is ready for downstream applications including NGS, microarrays, automated fluorescent DNA sequencing, restriction enzyme digestion, and other applications.

When performing Auto-Mag® PCR-Pure protocol, a magnet or centrifuge is required to pellet the magnetic particles. If performing the protocol manually without access to a magnet, sample tubes or plate 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).

Features:

- Rapid and reliable post-PCR and post enzymatic reaction clean-up
- High recovery of amplicons or dsDNA fragments greater than 100bp
- Single or double size selection capability and uniform fragments size distribution for NGS
- Adaptable to high throughput liquid handling workstations
- Designed for "bottle swap" with no protocol change against major competitor
- Highly cost-effective, and available in bulk with volume discounts

Kit Contents

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

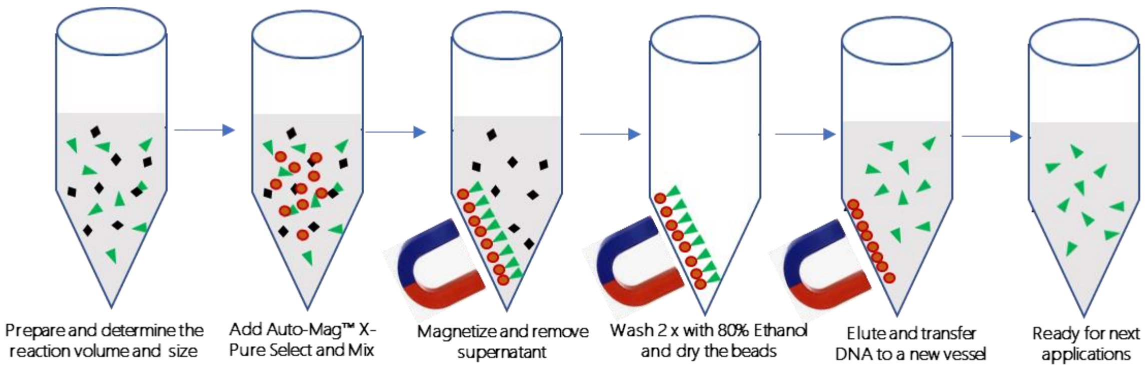
Auto-Mag® PCR-Pure is shipped at room temperature and is stable for at least 12 months from the date of purchase when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Additional Information

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

Illustrated Protocol for PCR amplicon clean-up



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Auto-Mag® PCR-Pure Protocol 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, such as library construction, next generation sequencing, Sanger sequencing, cloning, restriction digestions, adapter ligation, microarrays, and so on.

Materials and Equipment to Be Supplied by User

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) from each product supplier.

- Single-tube format: Nuclease-free 1.5 ml microcentrifuge tube, and Magnetic Rack Separator for 1.5 ml microcentrifuge tube
- 96-well format: 300ul round bottom plate for 1-100μl volume reaction and appropriate magnetic separation device for 96-well plate
- Centrifuge
- Sealing film for 96 well plate
- Vortex
- 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- RNase/DNase free Elution buffe (AMD-B232), TE Buffer, or Water (molecular biology grade)
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Bring the Auto-Mag® PCR-Pure reagent bottle to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Protocol for PCR Amplicon Clean-up

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

2. Conform the volume of PCR reaction samples and transfer the sample into a tube or well of 96-well plate.

3. Reference Table 5, Determine and add the appropriate volume of Auto-Mag® PCR-Pure reagent into a tube or the well of plate containing PCR reaction sample.

Table 5: Volume of PCR Reaction Sample and Suggested Volume of Auto-Mag® PCR-Pure Reagent

PCR Reaction Volume (μl)	Auto-Mag® PCR-Pure Volume Needed (μl) *

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10	18
20	36
25	45
50	90
100	180
Volume of Auto-Mag® PCR-Pure reagent per reaction = 1.8 X PCR reaction sample volume.	

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

5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 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: 80% ethanol must be freshly made.

7. Repeat steps 6 for second 80% ethanol wash and ensure all ethanol has been removed.

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 reduce the yield.

9. Remove the sample tubes or plate from the magnet. Add 20-50µl of elution buffer and mix by pipetting up and down 20 times. Ensure beads are no longer attached to the side of the well.

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

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

11. Place the sample tubes or plate back on the magnetic separation device 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.

Protocol for Removing Adapter Dimers

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. Reference Table 6, Determine and Add 0.8X volume of Auto-Mag® PCR-Pure reagent into the tubes or the well of reaction plate containing samples.

Table 6: Volume of adapter addition reaction and suggested Volume of Auto-Mag® PCR-Pure Reagent

Adapter addition reaction Sample Volume (µl)	Auto-Mag® PCR-Pure Volume Needed (µl) *
20	16
25	20
35	28
50	40
65	52
Volume of Auto-Mag® PCR-Pure reagent per reaction = 0.8 X sample volume.	

4. Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting 10 times or vortex for 10 seconds. Incubate at room temperature for 5 minutes.

5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 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: 80% ethanol must be freshly made.

7. Repeat steps 6 for second 80% ethanol wash and ensure all ethanol has been removed.

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 reduce the yield.

9. Remove the sample tubes or plate from the magnet. Add 20-50µl of elution buffer and mix by pipetting up and down 20 times. Ensure beads are no longer attached to the side of the well.

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

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

11. Place the sample tubes or plate back on the magnetic separation device and wait 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.

Protocol for gDNA Clean-up

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

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

3. Reference Table 7, Determine and Add 1 X volume of Auto-Mag® PCR-Pure into a tube or the well of reaction plate containing samples.

   Table 7: Volume of gDNA Sample and Suggested Volume of Auto-Mag® PCR-Pure Reagent

gDNA Sample Volume (µl)	Auto-Mag® PCR-Pure Volume Needed (µl) *
10	10
20	20
30	30
40	40
50	50
Volume of Auto-Mag® PCR-Pure reagent per reaction = 1.0X gDNA sample volume.	

4. Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting 10 times or vortex for 10 seconds. Incubate at room temperature for 5 minutes.

5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 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: 80% ethanol must be freshly made.

7. Repeat steps 6 for second 80% ethanol wash and ensure all ethanol has been removed.

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 reduce the yield.

9. Remove the sample tubes or plate from the magnet. Add 20-50µl of elution buffer and mix by pipetting up and down 20 times. Ensure beads are no longer attached to the side of the well.

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

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

11. Place the sample tubes or plate back on the magnetic separation device 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 Protocol for DNA Size Selection

Auto-Mag® PCR-Pure reagent can be used to speed and simplify nucleic acid size selection for fragment library preparation for Next Generation Sequencing. In this process, the desired size cutoffs can be achieved by varying the volume/volume ratio of the Auto-Mag® PCR-Pure reagents added to the starting sample. The protocols provided below can be used to optimize the desired size selection range: such as left, right, or double size selection. Used manually or automated on a liquid handling system, Auto-Mag® PCR-Pure will provide rapid and consistent size selection suitable for most applications.

Materials and Equipment to Be Supplied by User

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) from each product supplier.

- Single-tube format: Nuclease-free 1.5 ml microcentrifuge tube, and Magnetic Rack Separator for 1.5 ml microcentrifuge tube
- 96-well format: 300ul round bottom plate for 1-100µl volume reaction and appropriate magnetic separation device for 96-well plate
- Centrifuge
- Sealing film for 96 well plate
- Vortex
- 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Elution buffe (AMD-B228 or 10 mM Tris, pH 8.0) TE Buffer, or Water (molecular biology grade))
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Bring the Auto-Mag® PCR-Pure reagent bottle to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent 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, the 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.

Protocol for Left-Side Size Selection

As a rule, increasing the ratio of Auto-Mag® PCR-Pure volume to sample volume will increase the efficiency of binding smaller DNA fragments. This Left-Side Size Selection protocol is suitable for the purification of DNA fragments of one selected size and greater; all fragments below the cut-off size will be excluded and all fragments above the cut-off size will be selected. Following procedure is an example left-side size selection protocol based on a 50µl input sample volume.

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. Based on the desired fragment sizes, reference Table 1, or using the calculation below, to determine the volume of Auto-Mag® PCR-Pure reagent that will be added to the reaction,

Table 1: The Required Volumes of Auto-Mag® PCR-Pure Reagent and Ratios for Left Side Size Selective Purification of DNA Populations of Various Sizes, Based on a 50µl Sample Volume.

Sample Volume (µl)	Approximate Size Cutoff (bp)	Auto-Mag® PCR-Pure Volume Needed * (µl)	Ratio of Auto-Mag® PCR-Pure to Sample Volume (v/v)
50	100	90	1.8X
50	150	75	1.5X
50	250	47.5	0.95X
50	350	37.5	0.75X
50	450	32.5	0.65X
50	550	31.25	0.625X
50	650	30	0.6X
50	800	27.5	0.55X
50	1000	25	0.5X
*Calculation formula: Volume of sample * ratio = volume of Auto-Mag® PCR-Pure* *Example: for 350bp left side select, 50µl sample * 0.75= 37.5µl of Auto-Mag® PCR-Pure*			

4. Add the corresponding volume of Auto-Mag® PCR-Pure to DNA samples, and mix well by pipetting 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes.

   Note: Insufficient mixing of sample and Auto-Mag® PCR-Pure will lead to inconsistent size selection results. Make sure to mix well.

5. Place the sample tubes or plate on a compatible magnetic separation device for 3-5 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. Remove and discard all of liquid. Do not disturb the attracted beads.

   Note: 80% ethanol must be freshly made.

7. Repeat steps 6 for a second 80% ethanol wash and ensure all ethanol has been removed.

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 reduce the yield.

9. Remove the sample tubes or plate from the magnet. Add 20-50µl of elution 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 more or less Elution Buffer to elute the sample. 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.

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

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

11. Place the sample tubes or plate back on the magnetic separation device 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.

Protocol for Right-Side Size Selection

This Right-Side Size Selection protocol is suitable for the purification of DNA fragments of a selected size and smaller. To perform Right-Side Size Selection, add the appropriate ratio of Auto-Mag® PCR-Pure reagents to the sample. This binds the larger fragments to the right of the target range that are to be discarded, while the smaller fragments to the left of the target range are in the supernatant which need to be moved to a fresh tube. A re-bind step is used to exchange the buffer by adding additional Auto-Mag® PCR-Pure reagents into the supernatant to bind all the desired fragments. Although the ratio for the re-bind step can be altered to fit the application, 1.8x, and 1.2x, are commonly used ratios. The following is an example protocol based on a 50µl input sample volume and a 1.8x ratio for the re-bind step.

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. Based on the desired fragment size for the upper cut-off, Reference Table 2, or using the calculation below, to determine the needed volume of Auto-Mag® PCR-Pure reagent that will be added to the reaction.

Table 2. The Required Volumes of Auto-Mag® PCR-Pure Reagent and Ratios for First Step of Right Side

Selective Purification of DNA Populations of Various Sizes, Based on a 50µl Sample Volume

Sample Volume (µl)	Approximate Size Cutoff (bp)	Volume of Auto-Mag® PCR-Pure Needed for Desired Size DNA Exclusion. (µl)	Ratio of Auto-Mag® PCR-Pure to Sample Volume (v/v)
50	250	47.5	0.95X
50	350	37.5	0.75X
50	450	32.5	0.65X
50	550	31.25	0.625X
50	650	30	0.6X
50	800	27.5	0.55X
*Calculation formula: Volume of sample * ratio = volume of Auto-Mag® X-Pure Select* *Example: for 350bp cutoff, 50µl * 0.75x ratio = 37.5µl of Auto-Mag® PCR-Pure*			

4. Add the corresponding volume of Auto-Mag® PCR-Pure to DNA samples, and mix well by pipetting 10 times or vortexing for 10 seconds. Incubate at room temperature for 5 minutes.

   Note: Insufficient mixing of sample and Auto-Mag® PCR-Pure will lead to inconsistent size selection results. Make sure to mix well.

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

   Note: Fragments which are above the desired size range are bound to the Auto-Mag® PCR-Pure and are discarded.

6. Transfer the clear supernatant, which contains the Right-Side Size Selected DNA, to new reaction tubes or plate. Avoiding the transfer of any Auto-Mag® PCR-Pure beads.

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

7. Reference Table 3, or using the calculation below, to determine the additional volume of Auto-Mag® PCR-Pure reagent that will be added to the supernatant from Step 6 above.

Table 3. Required Additional Volume of Auto-Mag® PCR-Pure Reagent for Right Side Size Selection, Based on a 50µl Input Sample Volume and a 1.8x Ratio for The Re-Bind Step.

Original Sample Volume (µl)	Approximate Size Cutoff on First Step (bp)	Volume of Auto-Mag® PCR-Pure Needed for Re-bind Step. (µl)	Ratio of Auto-Mag® PCR-Pure to Sample Volume (v/v)
50	250	42.5	0.85X (total 1.8x)
50	350	52.5	1.05X (total 1.8x)
50	450	57.5	1.15X (total 1.8x)
50	550	58.75	1.175X (total 1.8x)
50	650	60	1.2X (total 1.8x)
50	800	62.5	1.25X (total 1.8x)
*Calculation formula: Sample volume * (1.8 – Initial ratio at step 3) = volume of Auto-Mag® X-Pure Select* *Example: for 350bp right-side select: 50µl * (1.8-0.75) = 52.5 µl of Auto-Mag® X-Pure Select*			

8. Add the corresponding volume of Auto-Mag® PCR-Pure to the supernatant, and 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 a compatible magnetic separation device for 3-5 minutes or until the magnetic beads are 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 a second 80% ethanol wash and ensure all ethanol has been removed.

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 20-50µl of elution 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 more or less Elution Buffer to elute the sample. 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.

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

15. Place the sample tubes or plate back on the magnetic separation device 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.

Protocol for Double Size Selection

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, the protocol requires an initial upper cut-off to exclude fragments that are too large, and a subsequent lower cut-off to exclude fragments that are too small. 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 4.

Table 4: Volumes and Ratios for Double Size Selective Purification of DNA Populations of Various Sizes, Based on a 50µl Sample.

Original Sample Volume (µl)	Approximate Target Peak Size (bp)	Volume and Ratios of Auto-Mag® PCR-Pure for Upper Cut-off		Volume and Ratios of Auto-Mag® PCR-Pure for Subsequent Lower Cut-off		Total Ratio
		Volume (µl)	Ratio	Volume (µl)	Ratio	
50	250	32.5	0.65X	15	0.3X	0.95X
50	300	30	0.60X	15	0.3X	0.90X
50	350	29	0.58X	12.5	0.25X	0.83X
50	400	27.5	0.55X	11.5	0.23X	0.78X
50	450	26.5	0.53X	10	0.20X	0.73X
50	500	25	0.50X	10	0.20X	0.70X
50	550	24.5	0.49X	8	0.16X	0.65X

Following is an example protocol of a 50µl input sample volume and a population of dsDNA fragments with a peak size at 300 bp. To adjust the size range and center point of the final population, refer to Table 4.

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. For remove unwanted larger DNA fragments, first add 30µl of well dispensed Auto-Mag® PCR-Pure reagent to the sample. (0.6X (v/v) ratio of Auto-Mag® PCR-Pure reagent to sample volume)

   Note: For a size-selective cutoff other than 300bp, refer to Table 4.

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

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

6. Keep the sample plate 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 transfer the supernatant. Significant bead transfer will cause tailing into the larger size range.

7. Add additional 15µl of well dispersed Auto-Mag® PCR-Pure reagent into the supernatant sample from step 6 (0.3x (v/v) ratio of Auto-Mag® PCR-Pure reagent to starting sample volume)

   Note: The total volume ratio of Auto-Mag® PCR-Pure reagent suspension to the original sample is 0.9 x now. ((30µl + 15µl) / 50µl=0.9x). For a size-selective cutoff other than 300bp, refer to Table 4.

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 magnetic separation device for 3-5 minutes, or until the Auto-Mag® X-Pure Select 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 11 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 20-50µl of elution 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 more or less Elution Buffer to elute the sample. 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.

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

15. Place the sample tubes or plate back on the magnetic separation device 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.

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 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

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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