AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® X-Pure Select

Version 2.1

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

Catalog Number: S003-01, S003-02, S003-03, S003-04

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® X-Pure Select is based on paramagnetic bead technology, designed for DNA fragment size selection of fragment library preparation for next generation sequencing (NGS). In the DNA size selection, size selection is required to produce a uniform distribution of fragments around an average size. The protocols of DNA size selection describe procedures of DNA size selection by adjusting volume ratio of Auto-Mag® X-Pure Select reagent to sheared DNA sample and the desired size DNA fragment are selected and recovered by a simple binding, washing and elution steps.

Auto-Mag® X-Pure Select also can be used for PCR amplicon, DNA fragment purification, the purification protocols provide the procedures for PCR purification in single tube or 96/384 well format. Auto-Mag® X-Pure Select reagent utilizes an optimized buffer to selectively bind DNA fragments >100 bp to the beads. Excess primers, nucleotides, salts, and enzymes can be removed using a simple washing procedure and PCR products are eluted with low salt elution buffer or water.

Auto-Mag® X-Pure Select is suitable for both manual and fully automated processing and the highly purified DNA can be used directly for downstream applications.

Features:

- Single or double size selection capability and uniform fragments size distribution for NGS
- High recovery of amplicons or dsDNA fragments greater than 100bp
- Uniform fragments size distribution
- 96- or 384-well formats
- Adaptable to high throughput liquid handling workstations
- Designed for “bottle swap” with no protocol change against major competitor.
- Significant cost savings

Kit Contents

Product Number	S003-01	S003-02	S003-03	S003-04
Auto-Mag® X-Pure Select	5 ml	50 ml	250 ml	500 ml

Storage and Stability

Auto-Mag® X-Pure Select 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	Fragmented DNA, PCR product
Starting amount	Scalable
DNA recovery	>90% recovery for DNA >100bp
Downstream Application	NGS, PCR, Cloning, Nucleic Acid Labeling, Mutation detection, genotyping, etc.
Processing format	Automated or 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 purification processing. 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 pellet the magnetic particles and discards the liquid. All processes are to be carried out at room temperature (15–25 °C).

Auto-Mag® X-Pure Select Protocols for DNA Size Selection & Cleanup

Auto-Mag® X-Pure Select can selectively bind DNA fragments based on the ratio of Auto-Mag® X-Pure Select 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 for fragment library preparation of next-generation sequencing (NGS)

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).
- 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 Molecular biology grade water, Tris (10 mM, pH 8.0) or TE (10 mM Tris, pH 8.1 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® X-Pure Select reagent bottle to room temperature for at least 30 minutes before use.
- Shake thoroughly the Auto-Mag® X-Pure Select reagent to fully resuspend the magnetic beads.

Sample preparation.

- DNA samples should be fragmented double-stranded DNA and dissolved in molecular biology grade water or lower salt buffer solution.
- 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.

Protocol for fragmented DNA Double Size Selection

For NGS library preparation, sheared DNA fragments need to be cleaned up and selected appropriate size. To perform Double Size Selection, the upper cut-off is performed first to remove larger fragments and then, the subsequent lower cut-off binds all fragments greater than the specified size to the beads whilst unwanted smaller fragments remain in the supernatant and are discarded. At this point, fragments within the desired range are bound to the beads and recover after the standard washing and elution steps.

To determine the DNA fragment selection range and corresponding ratio of Auto-Mag® X-Pure Select reagent, refer to Table 1.

Table 1: Reference conditions for DNA Double Size Selective for NGS library preparation

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® X-Pure Select / DNA	1.0x	0.9x	0.8x	0.7x	0.6x	0.55x	0.5x
2nd ratio of Auto-Mag® X-Pure Select / 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

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

2. Transfer 50µl sheared DNA sample into a 1.5-2.0 ml RNase-DNase free tube or well of 96-well plate. If sample is less than 50µl, add reagent grade water, or 10mM Tris-HCl pH8.0, to ensure the starting volume is at least 50µl.

3. Reference Table 1, first add the required volume of Auto-Mag® X-Pure Select reagent to the sample for remove unwanted larger DNA fragment.

   *Note: Volume of sample * ratio = volume of Auto-Mag® X-Pure Select reagent*
   *For example: for 250-320bp fragment select, 50µl sample * 0.8 ration = 40µl of Auto-Mag® X-Pure Select reagent*

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

   Note: Insufficient mixing will lead to inconsistent size selection results. Make sure to mix well. Pipette mixing is preferable as it tends to be more reproducible. If a 96 well plate and vortex is used, the plate must be sealed with a plate seal before vortex.

5. Incubate samples at room temperature for 5 minutes.

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until the solution is completely clear. The Auto-Mag® X-Pure Select bead settles to the magnet.

   Note: The higher sample volume and higher Auto-Mag® X-Pure Select ratio will require a longer settling time.

7. With the sample tube or plate on the magnet, transfer clear supernatant, which contains the Size Selected sample, into a new tube or plate. The reaction tube or plate with the remaining beads can be discarded.

Note: Do not disturb the attracted magnetic beads while transferring the supernatant. As the undesired larger fragment sizes are associated with the beads, significant bead transfer will cause tailing into the larger size range.

8. Reference Table 1, add an additional required volume of Auto-Mag® X-Pure Select reagent to the supernatant from step 6. This will bind the DNA fragments in the supernatant to the new Auto-Mag® X-Pure Select beads.

   *Note: Volume of sample * 0.2 ratio = volume of Auto-Mag® X-Pure Select reagent*
   *For example: for 250-320bp fragment select, 50µl sample * 0.2 = 10µl of Auto-Mag® X-Pure Select reagent. The total ratio of Auto-Mag® X-Pure Select reagent suspension to the original sample is 1.0 x now. (40µl +10µl) / 50µl=1.0x.*

9. Mix the Auto-Mag® X-Pure Select reagent and the samples thoroughly by pipette 10 times or vortex for 10 seconds.

   Note: Insufficient mixing will lead to inconsistent size selection results. Make sure to mix well. Pipette mixing is preferable as it tends to be more reproducible. If a 96 well plate and vortex is used, the plate must be sealed with a plate seal before vortex.

10. Incubate samples at room temperature for 5 minutes.

11. Place the sample tube or plate on a compatible magnetic separation device for 2 minutes or until the solution is completely clear. The Auto-Mag® X-Pure Select bead settles to the magnet.

    Note: The higher sample volume and higher Auto-Mag® X-Pure Select ratio will require a longer settling time.

12. Remove and discard the clear supernatant, which contains the unwanted smaller DNA fragments.

    Note: Do not disturb the attracted magnetic beads. As the desired library is associated with the beads, any bead loss during this step will result in reduced yield.

13. With the samples still on the magnet, add 180µl of 80% ethanol to each sample and incubate at room temperature for 30 seconds. Do not disturb the attracted beads. Mixing is not necessary.

14. With the sample still on the magnet, Remove and discard the ethanol supernatant by pipetting. Do not disturb the attracted beads.

15. Repeat steps 13-14 for the second 80% ethanol wash.

16. Dry the beads by incubating the plate at room temperature for 5 minutes with the sample 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 reduce the yield.

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

    Note: Depending on the downstream application, you can add any volume of Elution Buffer to elute the DNA. However, Elution volume should be large enough so that the liquid level is high enough for the beads to settle to the magnet.

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

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

20. Transfer the eluate (size selected DNA fragments) to an appropriate storage vessel and keep at -20°C for long term storage or for subsequent applications.

Protocol for removing adapter dimers from NGS library preparation samples

In the NGS library preparation, Adapter dimers contain full-length adapter sequences that are able to bind and cluster on the flow cell and generate sequencing data. they can negatively impact sequencing data quality and may even cause a run to stop prematurely. If adapter dimers are present in the library, it need to perform an additional clean-up step.

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

2. Conform the sample volume, Transfer sample into a 1.5-2.0 ml RNase-DNase free tube or well of 96-well plate.

3. Reference Table 2, Add 0.9x volume of Auto-Mag® X-Pure Select reagent to the adapter addition reaction sample.

Table 2: Some common adapter addition reaction volumes and suggested Auto-Mag® X-Pure Select volumes.

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

4. Mix the Auto-Mag® X-Pure Select reagent and the samples thoroughly by pipette 10 times or vortexing for 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 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 tubes or plate from the magnet. Add >20µl of elution buffer (reagent grade water, or TE buffer) and mix by pipet up and down 10 times.

    Note: Depending on the downstream application, you can add any volume of Elution Buffer to elute the DNA. However, Elution volume should be large enough so that the liquid level is high enough for the beads to settle to the magnet.

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 Cleanup: 96 Well Format, or Single-Tube Format

1. Completely float the Auto-Mag® X-Pure Select 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 3, add the appropriate volume of Auto-Mag® X-Pure Select reagent to the PCR sample.

Table 3: PCR sample volumes and suggested Auto-Mag® X-Pure Select volumes.

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

4. Mix the Auto-Mag® X-Pure Select 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.

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 purification	Recovery was measured by Spectrophotometry Absorbance. This 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® X-Pure Select	S003-01 S003-02 S003-03 S003-04	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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