AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® X-Pure Select

Version 1.0

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

S003-01	5 ml
S003-02	50 ml
S003-03	500 ml
S003-04	1000 ml

Contents

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.

Trademarks

Auto-Mag® X-Pure Select

Product Introduction

Auto-Mag® X-Pure Select consists of AMD's own paramagnetic beads and optimized chemicals that is designed for high-throughput clean up PCR, DNA fragments, RNA, 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® X-Pure Select can selectively bind fragments based on the ratio of Auto-Mag® X-Pure Select reagent to sample. Altering the ratio gives the user the ability to selectively keep or discard undesired fragment sizes. Auto-Mag® X-Pure Select 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® X-Pure Select 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:

- Single or double size selection capability and uniform fragments size distribution for NGS
- Rapid and reliable post-PCR and post enzymatic reaction clean-up
- High recovery of amplicons or dsDNA fragments greater than 100bp
- 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	S003-01	S003-02	S003-03	S003-04
Auto-Mag® X-Pure Select	5 ml	50 ml	500 ml	1000 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.

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. Working in RNase Free Conditions

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Some general precautions should be followed to avoid the introduction of contaminating nucleases especially during wash and elution steps. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions, and glassware. The following procedures should be followed to limit RNase contamination when working with RNA:

- Always wear gloves while working and change gloves frequently
- Refrain from using reagents, consumables and equipment that are in common use for other general lab processes
- Use dedicated RNase free equipment such as pipettes, pipette tips, gels boxes, etc.
- Work in a separate room, fume hood or lab space if available
- Use plastic, disposable consumables that are certified RNase free
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contamination of the stock solution
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work
- Keep the RNA on ice after extraction and while working with it.
- Store the extracted RNA at -20°C. For long term stability, keep the RNA at -80°C.

Auto-Mag® X-Pure Select Protocol for DNA Size Selection

Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent bottle to room temperature for at least 30 min before use.
- Suspension Auto-Mag® X-Pure Select 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.

A. Protocol for Left-Side Size Selection

As a rule, increasing the ratio of Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent that will be added to the reaction,

Table 1: The Required Volumes of Auto-Mag® X-Pure Select 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® X-Pure Select Volume Needed * (µl)	Ratio of Auto-Mag® X-Pure Select 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® X-Pure Select* *Example: for 350bp left side select, 50µl sample * 0.75= 37.5µl of Auto-Mag® X-Pure Select*			

4. Add the corresponding volume of Auto-Mag® X-Pure Select 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® X-Pure Select 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.

B. 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® X-Pure Select 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® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent that will be added to the reaction.

Table 2. The Required Volumes of Auto-Mag® X-Pure Select 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® X-Pure Select Needed for Desired Size DNA Exclusion. (µl)	Ratio of Auto-Mag® X-Pure Select 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® X-Pure Select*			

4. Add the corresponding volume of Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent that will be added to the supernatant from Step 6 above.

Table 3. Required Additional Volume of Auto-Mag® X-Pure Select 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® X-Pure Select Needed for Re-bind Step. (µl)	Ratio of Auto-Mag® X-Pure Select 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® X-Pure Select 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.

C. 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® X-Pure Select for Upper Cut-off		Volume and Ratios of Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent to the sample. (0.6X (v/v) ratio of Auto-Mag® X-Pure Select 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® X-Pure Select reagent into the supernatant sample from step 6 (0.3x (v/v) ratio of Auto-Mag® X-Pure Select reagent to starting sample volume)

   Note: The total volume ratio of Auto-Mag® X-Pure Select 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.

Auto-Mag® X-Pure Select Protocols for PCR, DNA, or RNA Clean-up

Auto-Mag® X-Pure Select offers a fast and convenient way to cleanup PCR products, DNA, RNA or other enzymatic reactions with high recovery rates. Auto-Mag® X-Pure Select 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® X-Pure Select reagent bottle to room temperature for at least 30 min before use.
- Suspension Auto-Mag® X-Pure Select by vortexing.

D. Protocol for PCR Amplicon Clean-up

1. Completely float the Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select Reagent

PCR Reaction Volume (µl)	Auto-Mag® X-Pure Select Volume Needed (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 sample volume.	

4. Mix thoroughly the Auto-Mag® X-Pure Select 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.

E. Protocol for Total RNA Clean-up

This protocol is suitable for the purification of RNA from various reaction systems, such as RNA purification from library construction experiments, and is not suitable for direct extraction of RNA from cells or tissues.

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

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

3. Reference Table 6, Determine and add the appropriate volume of Auto-Mag® X-Pure Select reagent into the reaction vessels containing 10-100µl RNA sample.

Table 6: Volume of Total RNA Sample and Suggested Volume of Auto-Mag® X-Pure Select Reagent

Total RNA Sample Volume (µl)	Auto-Mag® X-Pure Select Volume Needed (µl) *
10	18
20	36
25	45
50	90
100	180
Volume of Auto-Mag® X-Pure Select reagent per reaction = 1.8 X RNA sample volume.	

4. Mix thoroughly the Auto-Mag® X-Pure Select 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 RNase/DNase free 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.

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 -80°C for long term storage or for subsequent applications.

F. Protocol for Large RNAs and Small RNAs Separation and Cleanup (96-well format)

The following protocol exemplifies for enriched RNA below 200nt into the "small RNA" fraction while RNA above 200nt is enriched in the "large RNA" fraction from the 50 µl Total RNA samples.

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

2. Transfer 50 µl RNA sample into a well of 96-well plate. If sample volume is less than 50 µl, bring sample volume up to 50µl with nuclease-free water or the Elution Buffer.

3. Label the plate as "Large RNAs". Add 50µl Auto-Mag® X-Pure Select reagent into the wells containing 50µl total RNA sample. Mix thoroughly by pipetting 10 times or vortexing for 10 seconds.

4. Incubate at room temperature for 5 minutes to allow the large RNAs bound to Auto-Mag® X-Pure Select beads.

5. Place the Large RNAs plate on a compatible magnetic separation device for 3-5 minutes or until the magnetic beads are completely cleared from solution.

6. With Large RNAs plate still on the magnet, transfer ~100µl clean supernatant (including the small RNAs) to the new plate and label this new plate as "Small RNAs".

7. If need to recover large RNAs, go to step 13, to processing RNA washing steps.

   Note: if there is no need for large RNAs, discard the large RNA plate.

8. To recover small RNAs, use the "Small RNAs" plate and continue to step 9.

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

10. Add 40µl Auto-Mag® X-Pure Select reagent and 50µl 100% isopropanol into the well of small RNAs plate containing the clean supernatant from step 6. Mix thoroughly by pipetting 10 times or vortexing for 10 seconds.

11. Incubate at room temperature for 5 minutes to allow the small RNAs bound to Auto-Mag® X-Pure Select beads.

12. Place the Small RNAs 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.

13. Processing RNA wash steps

14. With the plate still on the magnet, add 250µl 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.

15. Repeat steps 14 for second 80% ethanol wash and ensure all ethanol has been removed.

16. 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.

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

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

19. Place the sample plate back on the magnetic separation device and wait 2-5 minutes or until the magnetic beads are completely cleared from solution.

20. Transfer the eluate (the Large RNAs (> 200nt) from large RNAs plate; or Small RNAs (< 200nt), from small RNAs plate) to an appropriate storage vessel. Keep at -80°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® X-Pure Select	S003-01 S003-02 S003-03 S003-04	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
Auto-Mag® RNA Elution Buffer	B232-01 B232-02 B232-03	50 ml 250 ml 500 ml

100% satisfaction guarantee on all AMD Research products, or your money back.

AMD Biotech is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call: 404-290-5063