Auto-Mag® X-Pure Select

Version 1.0

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

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

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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® X-Pure Select consists of AMD's own paramagnetic beads and optimized chemicals and is specifically designed for PCR amplicons cleaning up, DNA fragments, and RNA purification, or sheared DNA size selection in the library construction process for next generation sequencing (NGS).

Auto-Mag® X-Pure Select is an RNase-free reagent and can be used for RNA purification and clean-up. The PCR purification process consists of removal of salts, primers, primer-dimers, dNTPs, enzymes, adapters, and adapter dimers. The highly purified DNA is eluted with low salt elution buffer or water which can be used directly for downstream applications. In DNA size selection process, based on the volume ratio of Auto-Mag® X-Pure Select reagent to sample, the desired size DNA Fragment are selected and recovered. 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 is compatible with most next generation sequencing platforms.

Intended use:

Auto-Mag® X-Pure Select 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® X-Pure Select purified products can be used in the following applications:

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

Auto-Mag® X-Pure Select is also suitable for high-throughput purification of RNA or cDNA from in vitro applications such as transcription, antisense RNA (aRNA) amplification and RNA and cDNA probe synthesis. The purified RNA product can be used in the following applications:

- PCR and RT-PCR,
- Probes for microarray,
- RNase protection assays,
- Transfection for RNAi experiments,
- cDNA synthesis and labeling. etc.

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 18 months from the date of manufacture 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. Working in RNase Free Conditions

RNA purity and integrity are 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, contaminated laboratory equipment, 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 in 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.

3. 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).

Auto-Mag® X-Pure Select: Protocols for PCR Purification

Auto-Mag® X-Pure Select 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® X-Pure Select 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 S003-01	Product Number S003-02	Product Number S003-03	Product Number S003-04
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

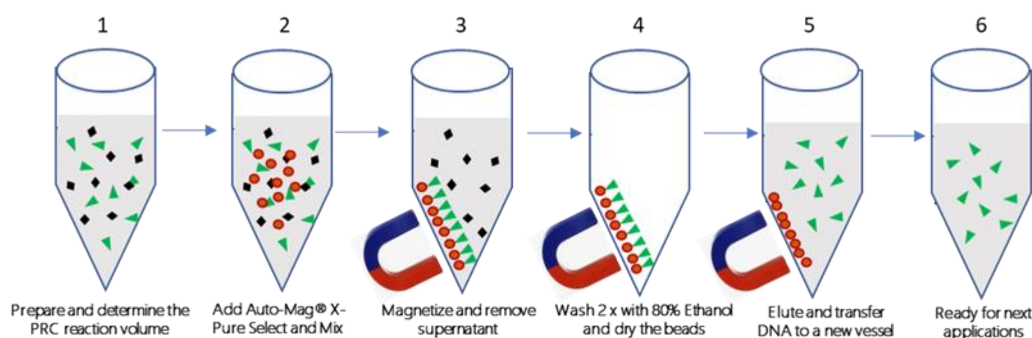
Materials and Equipment to Be Supplied by User

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

A. Procedure for PCR Amplicon Purification: 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. Add the appropriate volume of Auto-Mag® X-Pure Select reagent to the PCR sample reaction volume shown in Table 3.

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

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

B. Procedure for PCR Amplicon Purification: 384 Well Format

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

2. Confirm the volume of PCR reaction.

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

   Note: Generally, 384-well thermal cycling plates have a maximum well volume of 40 µl. Reactions of 14 µl or less can be purified in this type of plate (14 µl x 2.8 = 39.2 µl).

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

PCR Sample Reaction Volume (µl)	Auto-Mag® X-Pure Select Volume Needed at 1.8x (µl) *
5	9
7	12.6
10	18
14	25
(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 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 vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Place the reaction plate on a 384-well plate compatible magnetic separation device for 2-5 minutes or until the beads are completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

6. With the reaction plate still on the magnet, add 30µl 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 reaction plate 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 reaction plate from the magnet. Add 15-30µl 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 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 reaction plate 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® X-Pure Select: Protocols for RNA or cDNA Purification

Auto-Mag® X-Pure Select is suitable for high-throughput purification of RNA or cDNA from in vitro applications such as transcription, antisense RNA (aRNA) amplification and RNA and cDNA probe synthesis. Auto-Mag® X-Pure Select selectively binds RNA or cDNA to paramagnetic beads and excess oligonucleotides, nucleotides, salts, and enzymes can be removed using a simple washing procedure.

The RNA purification procedure is highly amenable to a variety of automation platforms because it utilizes magnetic separation and does not require centrifugation or vacuum filtration. Auto-Mag® X-Pure Select is not suitable for direct extraction of RNA from cells or tissues samples.

Working in RNase Free Conditions

RNA purity and integrity are 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 equipment, 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 in 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.

Materials and Equipment to Be Supplied by User

- Single-tube format: RNase-free 1.5-2.0 ml tube, and Magnetic Rack Separator for tube. (Any vendor of choice)
- 96-well format: Certified RNase/DNase-free 96 well thermal cycling plate, or 300µl round bottom microtiter plate, and appropriate magnetic separation device (Any vender of choice).

- 384-well format: Certified RNase/DNase-free 384 well (40µl well capacity) cycling plate and appropriate magnetic separation device (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 RNase/DNase free water, TRIS-Acetate (10 mM pH 8.0), or TE Buffer (10 mM Tris-Acetate pH 8.0, 1 mM EDTA) for RNA elution.
- Well calibrated pipettor and Disposable RNase/DNase free 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.
- Suspension Auto-Mag® X-Pure Select by vortexing.

C. Procedure for Total RNA or cDNA Purification: 96 Well Format

1. Completely float the Auto-Mag® X-Pure Select reagent until it appears homogeneous in color.
2. Confirm the volume of the RNA/cDNA samples and determine whether a plate transfer is necessary.

   Note: PCR plates generally have a maximum volume of 200 µl. If sample volume multiplied by 2.8 exceeds the volume of the PCR plate, transfer RNA/cDNA samples to a 300ul round plate.

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

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

Total RNA Sample Reaction Volume (µl)	Auto-Mag® X-Pure Select Volume Needed (µl) *
20	36
25	45
50	90
75	135
100	180
(Volume of Auto-Mag® X-Pure Select reagent per reaction) = 1.8 X (RNA reaction volume).	

4. Mix the Auto-Mag® X-Pure Select 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 RNA/cDNA products to the magnetic beads. Pipette mixing is preferable as it tends to be

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more reproducible. For reactions 50µl and larger, a longer incubation is recommended, and for the purification of single stranded cDNA, an incubation of up to 20 minutes can increase recovery. If vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Place the reaction 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: 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 and third 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 reduce the yield.

9. Remove the reaction plate from the magnet. Add 20-50µl of RNase free elution buffer (reagent grade water, or TE buffer) and pipette mix 20 times or seal to vortex 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.

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.

D. Procedure for Large Volume RNA or cDNA Purification: 96 Well Format

For large volume (>100 µl) RNA/cDNA sample purification, the process is a series of repeating Auto-Mag® X-Pure Select binding reactions/separation steps within the same well of Certified RNase/DNase-free microtiter plate until all volume of sample are processed.

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

2. Determine the total volume of RNA/cDNA samples and divide the volume so that each set of volume is less than 100µl, so the samples can be process in a well of 300µl round bottom microtiter plate.

   Note: for example: 180µl (total volume) / 2 = 90 µl (part volume of sample),

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255µl (total volume) / 3 = 85 µl (part volume of sample), etc.

3. Based on volume number calculated, transfer one part of the volume of sample into the well of 300µl capacity 96-well plates.

4. Based on the part the volume of sample, add the 1.8x volume of Auto-Mag® X-Pure Select reagent to the partial sample reaction volume. The volume of Auto-Mag® X-Pure Select for a given reaction can be derived from the following equation:

(Partial volume of sample) x 1.8 = (Volume of Auto-Mag® X-Pure Select per reaction)

Note: for example: For 90 µl (part volume) x 1.8 = 162 µl of Auto-Mag® X-Pure Select,
For 85 µl (part volume) x 1.8 = 153 µl of Auto-Mag® X-Pure Select, etc.

5. Mix the Auto-Mag® X-Pure Select 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.

6. Place the reaction plate on a compatible magnetic separation device for 5-10 minutes or until the magnetic beads are completely cleared from solution. Remove and discard all of liquid. Do not disturb the attracted beads.

   Note: The separation time is dependent on the size of the reaction. Larger reaction sizes will require more time for separation.

7. Dispense the next partial sample amount to the same well as the first part of the reaction., and repeat steps 4-6 until all volumes of the sample are processed before proceeding to the wash steps.

   Note: Some beads will be disturbed by the addition of the reaction. This is expected and will not cause a problem. Pipette mixing is preferable as it tends to be more reproducible. If vortexing is used, the plate must be sealed with a plate seal before vortexing. The color of the mixture should appear homogenous after mixing.

8. Keep the reaction plate on the magnet, add 280µl 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.

9. Repeat steps 8 for a second and third 80% ethanol wash.

10. 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.

11. Remove the reaction plate from the magnet. Add 20-50µl of RNase free elution buffer (reagent grade water, or TE buffer) and pipette mix 20 times or seal to vortex 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.

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

13. Place the sample plate or tubes back on the magnet and wait 2-5 minutes or until the magnetic beads are

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completely cleared from solution.

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

E. Procedure for Total RNA or cDNA Purification: Single-Tube Format

1. Completely float the Auto-Mag® X-Pure Select reagent until it appears homogeneous in color.
2. Confirm the volume of the RNA/cDNA samples and transfer the sample into 1.5-2.0 ml RNase-free tubes.
3. Add the appropriate volume of Auto-Mag® X-Pure Select reagent to the sample reaction volume shown in Table 6.

Table 6: Auto-Mag® X-Pure Select to Sample Reaction Volume Chart

Total RNA Sample Reaction Volume (µl)	Auto-Mag® X-Pure Select Volume Needed (µl) *
50	90
75	135
100	180
150	270
200	360
250	450
300	540
400	720
500	900
(Volume of Auto-Mag® X-Pure Select reagent per reaction) = 1.8 X (RNA reaction volume).	

4. Mix the Auto-Mag® X-Pure Select reagent and the samples thoroughly by pipette mixing 20. Incubate at room temperature for 5 minutes for maximum recovery.

Note: This step binds RNA/cDNA products to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. For larger reaction, a longer incubation is recommended, and for the purification of single stranded cDNA, an incubation of up to 20 minutes can increase recovery.

5. Place the reaction tube on a magnetic tube rack for 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 500-1000µl 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: The volume of ethanol required for washing will depend on the size of the original reaction. The wash solution must completely cover the entire bead mass on the side of the tube.

7. Repeat steps 6 for a second and third 80% ethanol wash.
8. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 10 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 reaction tube from the magnet. Add 30-50µl of RNase free elution buffer (reagent grade water, or TE buffer) and pipette mix 20 times. 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.

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

11. Place the sample tubes back on the magnet 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.

F. Procedure for Total RNA or cDNA Purification: 384 Well Format

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

2. Confirm the volume of RNA/cDNA samples.

3. Add the appropriate volume of Auto-Mag® X-Pure Select reagent to the sample reaction volume shown in Table 7.

Note: Generally, 384-well thermal cycling plates have a maximum well volume of 40 µl. Reactions of 14 µl or less can be purified in this type of plate (14 µl x 2.8 = 39.2 µl).

Table 7: Auto-Mag® X-Pure Select to Sample Reaction Volume Chart

Total RNA Sample Volume (µl)	Auto-Mag® X-Pure Select Volume Needed (µl) *
5	9
7	12.6
10	18
14	25
(Volume of Auto-Mag® X-Pure Select reagent per reaction) = 1.8 X (RNA sample volume).	

4. Mix the Auto-Mag® X-Pure Select 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 RNA/cDNA products to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. For the purification of single stranded cDNA, an incubation of up to 20 minutes can increase recovery.
If vortexing is used, the plate must be sealed with a plate seal before vortexing.

5. Place the sample plate on a compatible magnetic separation device for 5-10 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 30µl 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 and third 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 reduce the yield.

9. Remove the reaction plate from the magnet. Add 15-30µl of RNase free elution buffer (reagent grade water, or TE buffer) and pipette mix 20 times or seal to vortex 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.

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

11. Place the sample plate 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 -80°C for long term storage or for subsequent applications.

G. Procedure for Large RNAs and Small RNAs Separation and Cleanup: 96-well format

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

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

2. Transfer 50µl RNA sample into a well of 96-well plate. If sample volume is less than 50µl, bring sample volume up to 50µl with nuclease-free water or the RNA 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 pipette mixing 10 times or vortexing for 10 seconds.

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

4. Incubate reaction plate 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 containing the clean supernatant from step 6.

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. Mix thoroughly by pipette mixing 10 times or vortexing for 10 seconds.

11. Incubate the "Small RNAs" plate at room temperature for 10 minutes to allow the small RNAs bound to Auto-Mag® X-Pure Select beads.

12. Place the Small RNAs plate on the magnet 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 for the Large RNAs plate, or the Small RNAs plate, or both.

14. With the plate 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.

15. Repeat steps 14 for second and third 80% ethanol wash.

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 reaction plate from the magnet. Add 20-50µl of RNase free elution buffer (reagent grade water, or TE buffer) and pipette mix 20 times or seal to vortex 20 seconds. 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 magnet 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 appropriate storage vessel. Keep at -80°C for long term storage or for subsequent applications.

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.

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

H. Procedure for DNA 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,

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 8.

Table 8: 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® 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

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

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

4. Mix the Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent into the supernatant from step 6.

Note: The volume of Auto-Mag® X-Pure Select to add = volume of sample x 2nd ratio chosen. (10µl =50 µl x 0.2x) 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).

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® 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 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.

I. Procedure for NGS Library Size Selection

The Auto-Mag® X-Pure Select 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 9.

Table 9. recommended ratio of captured library DNA length to Auto-Mag® X-Pure Select

NGS Library Fragments to capture	Recommended Ratio	Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select reagent to the sample for removing unwanted larger DNA fragments.
4. Mix the Auto-Mag® X-Pure Select 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® X-Pure Select 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® X-Pure Select 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® 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 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 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 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

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