Auto-Mag® Micro-Select

Version 2.1

Magnetic beads-based reagent for small size DNA, ssDNA, Oligo, or RNA cleanup and size selection

Catalog Number: S004-01, S004-02,

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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® Micro Size Select is designed to recover and purify small DNA fragments as small as 15bp from sheared DNA fragments, PCR or RT-PCR reactions, and enzyme digestion. Another notable feature is the selection of small-sized DNA fragments. Like the DNA double-sided size selection steps, the small DNA fragments of a specific size or size range (15bp-300bp) can be recovered from the sample by adjusting the volume ratio of Auto-Mag® Micro size select to the DNA sample. The purified small DNA fragments can be used for PCR, fragment analysis, fluorescent or radioactive sequencing, capillary electrophoresis, DNA labeling and ligation, etc.

Auto-Mag® Micro Select is an RNase free reagent that can be used for miRNA enrichment and RNA size selection from total RNA.

Auto-Mag® Micro Size select uses magnetic technology, and it is suitable for manual procedures as well as for adapting it to automatic liquid handling workstations.

Features:

- Effective purification and recovery of short DNA and RNA samples
  - dsDNA fragments 15 bp or longer
  - ssDNA fragments 20 nt or longer
  - Oligo and chimeric oligo 20 nt or longer
  - RNA fragments 20 nt or longer
- Removal of impurities and unwanted reaction components
- Fragment size selection of short DNA and RNA samples for specific applications
  - DNA Fragment Size select ranger: 15bp up
  - RNA fragment 25nt -200nt
- Expectant recover Yield: Up to 95%
- Compatible with manual and automated processing and cost effective.

Kit Contents

Product Number	**S004-01**	**S004-02**
Preparations*	100	1,000
Auto-Mag® Micro Size Select	10 ml	100 ml
Elution Buffer	10 ml	100 ml
**The number of preparations is based on 50µl of sample volume*		

Storage and Stability

Auto-Mag® Micro Size Select is shipped at room temperature and is stable for 12 months when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Preparation of Reagents

1. Prepare 80% Ethanol wash buffer. (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 within one week.

Additional Information

1. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	Fragmented DNA, PCR product, Micro-RNA, Oligo, ssDNA, etc.
Starting amount	Scalable
DNA recovery	>90% recovery for DNA >15bp
Downstream Application	PCR, Sequencing, Nucleic Acid Labeling, Fragment analysis, etc.
Processing format	Automated or Manual
Storage	2°C - 8°C

Auto-Mag® Micro Size Select Protocols

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 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® Micro Size Select reagent to room temperature for at least 30 minutes before use.
- Shake thoroughly the Auto-Mag® Micro Size 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 standard buffer solution such as Tris or TE.
- For the best results, the sample volume should be ≥ 20µl. A lower volume will decrease pipetting accuracy, therefore increasing selection point variability.

Protocol for Small Size DNA Size Selection and Cleanup

To purify and selectively isolate small DNA fragments within the target range, it is necessary to first remove larger fragments that fall outside this range. This process involves two sequential separation steps.

In the first step, determine the appropriate volume of Auto-Mag® Micro Size Select based on the sample volume and the upper size limit of the target DNA fragments. Add the calculated reagent to the sample to selectively eliminate DNA fragments exceeding the upper size threshold. After magnetic separation, transfer the supernatant containing the target DNA fragments to a new tube.

In the second step, recalculate the required reagent volume to capture all target DNA fragments, then add it to the supernatant and mix thoroughly. Perform the washing and elution steps to recover DNA fragments within the desired size range. The specific recovery ranges and corresponding reagent volumes are detailed in the table below.

Table 1: Reference conditions for small DNA Size Selection

The DNA size range to recovery (bp)	<50bp	<75bp	<100bp	<200bp	<300bp
1st ratio of Auto-Mag® Micro Size select / DNA	1.2x	1.0x	0.8x	0.6x	0.4x
2nd ratio of Auto-Mag® Micro Size select / DNA	0.8x	1.0x	1.2x	1.4x	1.6x
Total ratio	2.0x	2.0x	2.0x	2.0x	2.0x

Following is an example procedure to selectively recovery <50bp DNA fragments from 50ul DNA fragments input.

1. Completely float the Auto-Mag® Micro Size select reagent until it appears homogeneous in color.

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

3. Reference Table 1, first add 60µl of Auto-Mag® Micro Size select reagent to the sample for removing unwanted larger DNA fragments.

   Note: The volume of Auto-Mag® Micro Size select to add = volume of sample x 1st ratio chosen. (60µl =50µl x 1.2)

4. Mix the Auto-Mag® Micro Size select reagent and the samples thoroughly by pipette mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 8 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 8 minutes, or until the Auto-Mag® Micro-Select bead is completely cleared from solution.

6. Keep the sample on the magnet, transfer ~110µl supernatant into new tube or the well of a new plate. Discard beads that contain 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 1, Add an additional 40µl of well dispersed Auto-Mag® Micro Size select reagent into the supernatant from step 6.

   Note: The volume of Auto-Mag® Micro Size select to add = volume of sample x 2nd ratio chosen. (40µl=50µl x 0.8) The total ratio of Auto-Mag® Micro Size select reagent suspension to the original sample is 2.0 x now. ((60µl + 40µl) / 50µl=2.0x).

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

9. Place the sample tubes or plate on the magnet for 8 minutes, or until the Auto-Mag® Micro Size select bead is completely cleared from solution. Remove and discard all of the 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 the liquid. Do not disturb the attracted beads.

    Note: 80% ethanol must be freshly made.

11. Repeat step 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 5 minutes or until the magnetic beads are completely cleared from solution.

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

Protocol for labeled DNA, RNA or oligonucleotide probes purification

To purify and recover labeled DNA, RNA, or oligonucleotide probes, add Auto-Mag® Micro Size Select reagent and 100% isopropanol, each at a volume equal to twice the sample volume. The labeled probes are then efficiently recovered through simple steps of separation, washing, and elution.

1. Completely float the Auto-Mag® Micro Size select reagent until it appears homogeneous in color.

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

3. Add 2X volume of Auto-Mag® Micro Size select reagent and 2X volume of 100% isopropanol to the sample.

Reference Table below, add the appropriate volume of Auto-Mag® Micro Size select and Isopropanol to the samples.

Volume of sample (µl)	**Volume of Auto-Mag® Micro Size select needed (µl)**	**100% Isopropanol needed (µl)**
10	20	20
20	40	40
25	50	50
50	100	100
(Volume of Auto-Mag® Micro Size select & Isopropanol per reaction) = 2 X (Volume of samples).		

4. Mix the Auto-Mag® Micro Size select reagent and the samples thoroughly by pipette mixing 10 times or vortexing for 10 seconds. Incubate at room temperature for 8 minutes for maximum recovery.

5. Place the sample tubes or plate on a compatible magnetic separation device for 8 minutes, or until the Auto-Mag® Micro-Select bead is completely cleared from solution.

6. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Keep the samples on the magnet, add 200µl of 80% ethanol to each sample and incubate for 30 seconds at room temperature.

8. Remove and discard all the liquid. Do not disturb the attracted beads.

   Note: 80% ethanol must be freshly made.

9. Repeat steps 7-8 for a second 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 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. 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.

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

13. 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.

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

Protocol for Small RNA Size Selection and Cleanup

To select and recover small RNA molecules, it is necessary to remove larger RNA first, and then to recover all small RNA, including miRNA, siRNA, and aRNA from pre-purified total RNA or enzyme reactions.

1. Completely float the Auto-Mag® Micro Size select reagent until it appears homogeneous in color.

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

3. Add exactly 25µl of Auto-Mag® Micro Size select reagent to the sample for removing unwanted larger RNA fragments.

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

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

5. Place the sample tubes or plate on a compatible magnetic separation device for 10 minutes, or until the Auto-Mag® Micro-Select bead is completely cleared from solution.

6. Keep the sample on the magnet, transfer ~75µl supernatant (including the small size RNAs) into new tube or the well of a new plate. Discard the beads that contain unwanted large RNA 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 25µl of well dispersed Auto-Mag® Micro Size select reagent and 25µl of 100% isopropanol into the supernatant from step 6.

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

9. Place the sample tubes or plate on the magnet for 10 minutes, or until the Auto-Mag® Micro Size select bead is completely cleared from solution. Remove and discard all of the 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 the liquid. Do not disturb the attracted beads.

    Note: 80% ethanol must be freshly made.

11. Repeat step 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 RNA. 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 -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 yield/ Incorrect recovery of purification		
	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.
RNA Size incompatible	Insufficient ratio	Use volume ratios outlined in this manual.
	Insufficient ethanol concentration used for washing step	Use freshly prepared 80% ethanol.
	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® Micro Size Select	S004-01	10 ml
	S004-02	100 ml
RNA Elution Buffer	B232-01	50 ml
	B232-02	250 ml
	B232-03	500 ml
Auto-Mag® RNA-Pure	S008-01	5 ml
	S008-02	50 ml
	S008-03	250 ml
	S008-04	500 ml

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