Auto-Mag® NGS Clean-up and Size selection Kit

Cat # AMD-S013 Version 2.0

❖ Magnetic beads-based reagent for quick high-throughput DNA size selection or clean-up during the next generation sequencing library preparation for all NGS platforms,

Highlights

- Designed for "bottle swap" with no protocol change against major competitor
- Double-sided size selection capability and uniform fragments size distribution
- Reliable cleanup from different reaction mixtures and buffers
- High recovery of amplicons or dsDNA fragments greater than 100bp. Complete removal of NGS adapters, primers, nucleotides, enzymes and other reaction components inhibiting NGS workflow
- No centrifugation or filtration needed. Manual and automation-friendly sample processing in 96- and 384-well formats

Protocol Contents

Disclaimers and Safety Information

This kit is designed for research purposes only. The all biological samples are considered potentially infectious. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

Trademarks

The trademarks mentioned herein are the property of AMD Biotech Inc. or their respective owners.

Product Introduction

Auto-Mag® NGS Clean-up and Size selection Kit utilizes AMD's unique paramagnetic bead technology for quick high-throughput DNA fragment clean up and size selection during the next generation sequencing library preparation for all NGS platforms, e.g. Illumina, PacBio, Solid, etc. This kit includes all the necessary reagents for the number of preparations listed and can be used for both manual and fully automated purification of DNA samples.

For the DNA size selection, the binding of DNA to magnetic beads is based on a ratio of Auto-Mag® PCR-Pure reagent and the original DNA solution. The more Auto-Mag® PCR-Pure reagent used in a reaction, the smaller the size of DNA selected. Following the protocol, the unwanted DNA fragment sizes of either larger or smaller than the DNA of interest is removed during the binding and rebinding steps. This kit can help users to effectively achieve a result of the desired size selection range from a population of DNA fragments.

Applications

- DNA fragment library cleanup and size-selection for downstream use in next generation sequencing workflows

Kit Contents

Product Number	AMD-S013-1	AMD-S013-10	AMD-S013-50
Number of Preparation*			
Auto-Mag® PCR-Pure	1.2 ml	11 ml	52 ml
DNA Wash Buffer	1.5 ml	10 ml	25 ml x 2
DNA Elution Buffer	1.5 ml	15 ml	60 ml

*The number of preps is based on the volume of DNA sample.

Storage and Stability

Auto-Mag® NGS Clean-up and Size selection Kit is shipped at room temperature and is guaranteed for at least 12 months from the date of purchase when the kit is stored at 2-8°C after received. Do not freeze at any time.

Preparation of Reagents

Prepare the following components for each kit before use:

- Dilute DNA wash Buffer with 100% ethanol as follows and store at room temperature

	100% Ethanol to be added
Kit	DNA Wash Buffer
AMD-S013-1	6 ml
AMD-S013-10	40 ml
AMD-S013-50	100 ml x 2

Protocol for DNA double-sided size selection (96-well Plate Format)

This protocol can be used to generate DNA fragment libraries with a certain size range or to narrow fragment-size distribution. Through a two times selections process, the both smaller and larger fragments can be removed and the expected size range of DNA fragments can be obtained. This method is called double-sided size selection. The following protocol exemplifies a size selection of DNA fragment libraries with a size range of 250–400bp from a 50ul DNA samples. The information provided is an approximate guide. By altering the volume ratios of DNA fragment libraries and PCR-Pure reagents, other size ranges can be obtained. The optimal ratio for other size ranges should be determined empirically.

Materials and Equipment to be supplied by User:

- 100% Ethanol
- For 96 well format: 96 well cycling plate
- Magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipette and Disposable pipette tips
- 80% ethanol for additional DNA Wash.

Before Starting

- Prepare DNA Wash Buffer according to Preparation of Reagents Section.
- If kit was stored at 2-8°C, bring the Auto-Mag® PCR-Pure reagent to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent by vortexing.

Procedure

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

2. For using the 96-well plate, a starting sample volume of 50µl is recommended. For smaller samples, add nuclease-free water to adjust the volume.

3. For removing the large DNA fragments (>400bp), first add 35µl of well dispensed Auto-Mag® PCR-Pure reagent into the wells containing 50µl DNA sample. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The volume ratio of Auto-Mag® PCR-Pure reagent suspension to the sample is 0.7x.

4. Incubate the mixture for 5 minutes at room temperature to allow the large DNA fragments are bound to Auto-Mag® PCR-Pure beads.

5. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear.

6. Keep the sample plate on the magnet and transfer the ~85µl of supernatant into the well of a new plate and discard the beads that contain the unwanted large DNA fragments.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

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

8. For the smaller DNA fragments cutoff (< 250bp), add 15µl of well dispersed Auto-Mag® PCR-Pure reagent into the well containing supernatants from step 6. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The total volume ratio of Auto-Mag® PCR-Pure reagent suspension to the original sample is 1.0 x now; (35µl and 15µl to 50µl).

9. Incubate the sample plate for 10 minutes at room temperature to allow the selected DNA fragment are bound to Auto-Mag® PCR-Pure beads.

10. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

11. Keep the sample plate on the magnet and add 200µl of DNA Wash Buffer or 80 % ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: DNA Wash Buffer must be diluted with ethanol prior to use.

12. Repeat Steps 11 for second DNA wash.

13. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

Note: It is critical to completely remove all traces of liquid but take caution in not over drying the beads as this will reduce the yield.

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer all of supernatant to a new 96-well plate or an appropriate storage vessel. The ultra-pure DNA fragments (250-400bp) are ready for next step of NGS library preparation process or store at -20°C.

Protocol for removing adapter dimers (96-well Plate Format)

This protocol can be used to remove un-ligated adapters and adapter dimers after an adapter addition reaction in NGS library construction.

Materials and Equipment to be supplied by User:

- 100% Ethanol
- For 96 well format: 96 well cycling plate
- Magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipette and Disposable pipette tips
- 80% ethanol for additional DNA Wash.

Before Starting

- Prepare DNA Wash Buffer according to Preparation of Reagents Section.
- If kit was stored at 2-8°C, bring the Auto-Mag® PCR-Pure reagent to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent by vortexing.

Procedure

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

2. Add 0.8 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100µl adapter addition reaction sample according to the instructions in the table below.

Table: Some common adapter addition reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

Adapter addition reaction Sample Volume (µl)	Auto-Mag® PCR-Pure Volume (µl)*
25	20
50	40
65	52
100	80

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for the sample: Auto-Mag® PCR-Pure reagent volume per reaction = 0.8 X sample volume.*

3. Mix thoroughly the Auto-Mag® PCR-Pure reagent and DNA sample by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

4. Place the sample plate on the 96 well magnetic separation device and allow magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of DNA Wash Buffer or 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: DNA Wash Buffer must be diluted with ethanol prior to use. Do not disturb the attracted beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second DNA wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating the sample plate at room temperature for 5 minutes.

Note: It is critical to completely remove all traces of liquid but take caution in not over drying the beads as this will reduce the yield.

8. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of Elution Buffer to each well and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

Note: Pre-warming the elution buffer at 55°C can increase the yield.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.

Note: If necessary, perform a second 0.8x bead-based clean-up.

Additional clean-up

11. Add 0.8 volume of Auto-Mag® PCR-Pure reagent to the supernatant collected from Step 10.

12 Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

13. Repeat Steps 4-7 (capture and washes)

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.

Protocol for NGS library clean-up (96-well Plate Format)

This protocol can be used for NGS library clean-up to remove contaminants (such as, nucleotides, primers, adapters, enzymes, buffer additives, salts) and shorter DNA fragments, less than150–200 bp from NGS library samples.

Materials and Equipment to be supplied by User:

- 100% Ethanol
- For 96 well format: 96 well cycling plate
- Magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipette and Disposable pipette tips
- 80% ethanol for additional DNA Wash.

Before Starting

- Prepare DNA Wash Buffer according to Preparation of Reagents Section.
- If kit was stored at 2-8°C, bring the Auto-Mag® PCR-Pure reagent to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent by vortexing.

Procedure

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

2. Add 1.0 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100µl DNA sample according to the instructions in the table below.

Table 3: Some common reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

DNA Sample Volume (µl)	Auto-Mag® PCR-Pure Volume (µl)*
10	10
20	20
25	25
50	50
100	100

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for DNA sample: Auto-Mag® PCR-Pure reagent volume per reaction = 1.0 X DNA sample volume.*

3. Mix thoroughly the Auto-Mag® PCR-Pure reagent and DNA sample by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

4. Place the sample plate on the 96 well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of DNA Wash Buffer to each well and incubate the sample plate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: DNA Wash Buffer must be diluted with ethanol prior to use. Do not disturb the attracted beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat Steps 5 for a second DNA wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating the sample plate at room temperature for 5 minutes.

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.

8. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50 µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 1-404-259-6276 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low DNA yield	Insufficient ratio	Use volume ratios outlined in this manual.
	Insufficient ethanol concentration used for washing step	Prepare DNA Wash Buffer by use freshly 100 % ethanol.
	Elution buffer volume insufficient	Bead pellet must be covered completely with elution buffer
	Beads over dried	Do not dry beads longer than 15 min 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 of the ethanol after the final washing step. Dry beads 5–10 min 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
	Aspiration speed too high (elution step)	High aspiration speeds during the elution step may cause bead carry-over. Reduce aspiration speed for elution step