Auto-Mag® DTR

Cat # AMD-S004 Version 2.0

Highlights

- Designed for "bottle swap" with no protocol change against major competitor
- Generate long contiguous sequence read lengths (>900 bases) with a low retest rate
- Efficient elimination of sequencing reaction contaminants and high DNA recovery yields
- Reduce Big-Dye usage
- No centrifugation or vacuum steps
- Utilize a rapid, simple protocol with minimal hands-on time and adaptable to common liquid handling workstations that allows you to process more samples for sequencing every day
- Available in a wide range of formats - single sample, 8-well, 96-well and 384-well formats Cost Effective.
- Save up to 80% to similar products.

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® DTR is a paramagnetic bead-based system designed for the removal of primer-dimers, salts, and unincorporated dye terminators from Sanger sequencing reaction mixtures. The high sensitivity and binding ability of Auto-Mag® DTR allows for decreased concentrations of BigDye® chemistry to be used and longer continuous read lengths to be achieved. Auto-Mag® DTR can be processed in 96- and 384-well formats with three simple steps: bind, wash, and elute. The protocol can be used for manual procedure as well as guideline for adapting it to automatic liquid handling workstations currently on the market (e.g. Beckman, Hamilton, Tecan, Beckman Courter, Caliper, Perkin Elmer, Agilent and Eppendorf)

Application

Clean up of sequencing product for both ABI and MegaBACE platforms

Kit Contents

Product Number	AMD-S004-5	AMD-S004-50	AMD-S004-250	AMD-S004-500
Auto-Mag® DTR	5 ml	50 ml	250 ml	500 ml
Number of Preparation*	500	5,000	25,000	50,000

*Number of reactions is based on 10µl reaction volume. 10µl of Auto-Mag® DTR is used regardless of the volume of the sequencing reaction

Storage and Stability

Auto-Mag® DTR 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. It should never be frozen at any time.

Protocol for Auto-Mag® DTR: 96-well Plate Format

Materials and Equipment to be supplied by User:

- 100% Ethanol (do not use denatured ethanol)
- 96-well plate capable of being used in sequencer
- Multichannel pipette
- Polypropylene reservoirs
- Magnetic separation device compatible with 96-well PCR plate or for 1.5 ml tubes.
- Elution buffer (diH_2O or low salt buffer)

Before Starting

- Prepare fresh 85% Ethanol, (Prepare from absolute ethanol. Do not use denatured alcohol).
- Thoroughly shake the Auto-Mag® DTR reagent to resuspend the beads before use.

Procedure

1. Bring Auto-Mag® DTR to room temperature. Shake thoroughly the Auto-Mag® DTR reagent to fully resuspend the magnetic beads.

2. Add 10µl Auto-Mag® DTR reagent to each sequencing reaction sample.

Note: Use 10µl of Auto-Mag® DTR regardless of the volume of the sequencing reaction.

3. Add freshly prepared 85% Ethanol volume according to the table below:

Note: Do not use denatured ethanol. Always prepare fresh 85% Ethanol.

Sequencing Reaction Volume (µl)	85% Ethanol (µl)
5	30
10	40
15	50
20	60

4. Mix well the sample, the Auto-Mag® DTR reagent and 85% Ethanol by pipetting up and down 10 times.

5. Place the sample plate on the 96-well magnetic separation device and allow the Auto-Mag® DTR beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Carefully remove and discard the cleared supernatant.

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

6. Keep the sample plate on the magnet and Add 100µl 85% Ethanol to each well and incubate for 1 minute at room temperature or until the magnetic beads is fully resettled.

Note: It is not necessary to resuspend the magnetic beads.

7. With the plate still on the magnet, remove and discard the cleared supernatant. Do not disturb the magnetic beads.

8. Repeat Steps 6-7 for a second 85% Ethanol wash.

9. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample plate at room temperature for 10 minutes.

Note: It is important to remove any residue liquid and to dry the magnetic beads before elution. Residual ethanol may interfere with downstream applications.

10. Remove the sample plate from the magnetic separation device. Add 40µl appropriate Elution Buffer (diH_2O) to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

11. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from solution.

12. Transfer 30-35µl of the eluate (cleared supernatant) to a new plate to be loaded on a sequencer.

Note: Do not carry over any magnetic beads when transfer the elution.

Protocol of Auto-Mag® DTR: 384-well Plate Format

Materials and Equipment to be supplied by User:

- 100% Ethanol (do not use denatured ethanol)
- 384-well plate capable of being used in sequencer
- Multichannel pipette
- Magnetic separation device compatible with 384-well PCR plate
- Elution Buffer (diH_2O)

Before Starting

- Prepare fresh 85% Ethanol, (Prepare from absolute ethanol. Do not use denatured alcohol).
- Polypropylene reservoirs
- Thoroughly shake the Auto-Mag® DTR reagent to resuspend the beads before use.

Procedure

1. Bring Auto-Mag® DTR to room temperature. Shake thoroughly the Auto-Mag® DTR reagent to fully resuspend the magnetic beads.

2. Add 5µl Auto-Mag® DTR reagent to each Sequencing reaction sample.

Note: Use 5µl of Auto-Mag® DTR regardless of the volume of the sequencing reaction.

3. Add freshly prepared 85% Ethanol volume according to the table below:

Note: Do not use denatured ethanol. Always prepare fresh 85% Ethanol.

Sequencing Reaction Volume (µl)	85% Ethanol (µl)
5	15
10	22

4. Mix well the sample, the Auto-Mag® DTR reagent and 85% Ethanol by pipetting up and down 10 times.

5. Place the sample plate on the 384-well magnetic separation device and allow the Auto-Mag® DTR beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Carefully remove and discard the cleared supernatant.

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

6. Keep the sample plate on the magnet and Add 30µl 85% Ethanol to each well and incubate the sample plate for 1 minute at room temperature or until the magnetic beads is fully resettled.

Note: It is not necessary to resuspend the magnetic beads.

7. With the sample plate still on the magnet, remove and discard the cleared supernatant. Do not disturb the magnetic beads.

8. Repeat Steps 6-7 for a second 85% Ethanol wash.

9. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample plate at room temperature for 10 minutes.

Note: It is important to remove any residue liquid and to dry the magnetic beads before elution. Residual ethanol may interfere with downstream applications.

10. Remove the sample plate from the magnetic separation device. Add 20µl appropriate Elution Buffer (diH_2O) to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

11. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from solution.

12. Transfer the eluate (cleared supernatant) to a new plate to be loaded on a sequencer.

Note: Do not carry over any magnetic beads when transfer the elution.

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
Dye terminator remain in the eluted DNA and caused blobs	Sequencing reaction supernatant is not removed completely	Make sure to remove any liquid drops from each well of the plate.
	Too much BigDye	Use less BigDye per reaction.
	Insufficient washing	During wash Steps 6-8, resuspend the magnetic beads to wash more effectively
Low Sequencing Signal	Ethanol concentration is not correct	Make sure to use correct volume of ethanol
	Low ethanol concentration	Check the ethanol concentration, use fresh ethanol.
	Magnetic beads are lost during the process	Make sure not to remove any magnetic beads during aspiration.