Auto-Mag® DTR

Version 2.1

Magnetic beads-based reagent for Removal of unincorporated terminators from Sanger sequencing reactions

Catalog Number: S001-01, S001-02, S001-03, S001-04

Contents

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® DTR is consists of AMD Biotech's own paramagnetic beads and compatible buffers and is designed to remove of unincorporated dye terminators effectively and reliably from Sanger sequencing reaction mixtures. The process consists of three simple steps including bind, wash and elute. While binding the sequencing product selectively to the paramagnetic beads, unincorporated dyes, nucleotides, salts, and primers will be removed during ethanol washes. This principle allows for elution of the pure Sanger Sequencing product in the elution buffer of choice. Auto-Mag® DTR can be used for manual procedures as well as guidelines for adapting it to automatic liquid handling workstations.

Features:

- No protocol changes against major competitor.
- Longer Phred 20 read lengths averaging over 800 bases with a low retest rate.
- Pass rates over 85% or higher
- Manual or adjustable to automated liquid handlers.
- Reduce BigDye usage, due to increased average signal strength.
- 96well - or 384-well formats
- Up to 90% significant cost savings compared to any similar products.

Application:

Clean up of sequencing product for both ABI and MegaBACE platforms Supported Chemistries

- BigDye* versions 1.0, 1.1, 2.0, 3.0 and 3.1
- DYEnamic ET

*BigDye is a registered trademark of Applied Biosystems

Kit Contents

Product Number	S001-01	S001-02	S001-03	S001-04
Auto-Mag® DTR	5 ml	50 ml	250 ml	500 ml
Number of reactions – 96 well format	500 rxns	5,000 rxns	25,000 rxns	50,000 rxns
Number of reactions – 384 well format	1,000 rxns	10,000 rxns	50.000 rxns	100,000 rxns

Storage and Stability

Auto-Mag® DTR is shipped at ambient temperature and is stable for at least 12 months after delivery when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Preparation of Reagents

1. Prepare 85% 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. Freshly prepare 85% ethanol then keep cover tight and use in one week.

Additional Information

1. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	ABI Big Dye Chemistry Cycle Sequencing Reactions
Starting Amount	5 - 20 µl
Binding capacity	Scalable
Downstream Application	Sanger Sequencing,
Elution Volume	30 µl or above
Processing format	Automated; Manual
Storage	2°C - 8°C

Auto-Mag® DTR: Protocols

Auto-Mag® DTR uses a simple 3 steps procedure: Bind-Wash-Elute. Auto-Mag® DTR that contains the binding buffer is added to the sequencing products sample, the mixture is applied to the magnet plate (magnet stand), unincorporated dyes, nucleotides, salts, and other contaminants. are washed off and pure DNA is eluted, ready to be used in subsequent applications.

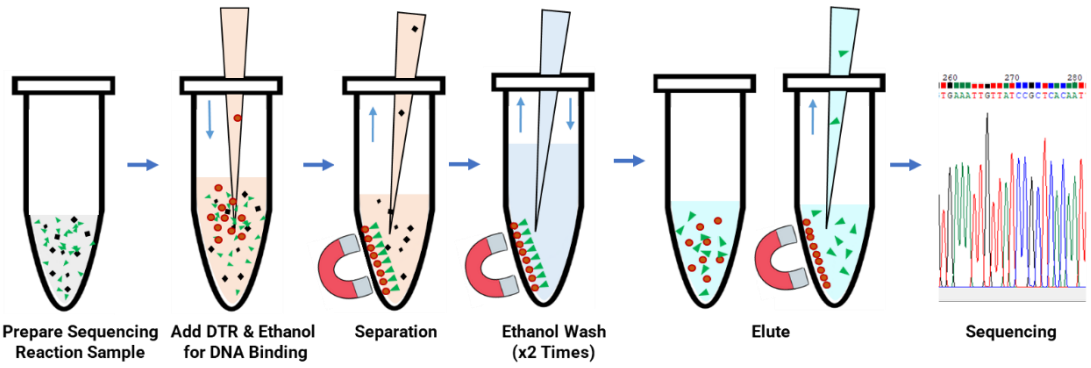
Materials and Equipment to Be Supplied by User

- 96-well format: 96-well cycling plate (Any vendor of choice).
- Magnetic separation device compatible with 96-well PCR plates
- 384-well format: 384-well cycling plate (Any vendor of choice).
- Magnetic separation device compatible with 384-well PCR plates
- Multichannel pipette
- Polypropylene Reservoirs
- Elution buffer (AMD-B101 or diH_2O, 0.1 mM EDTA, or low salt buffer)
- 96-well plate capable of being used in sequencers
- 85% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).

Before Starting

- Prepare 85% Ethanol for DNA wash steps according to the instructions of Preparation Reagents on page 3.
- Bring the Auto-Mag® DTR reagent bottle to room temperature for at least 30 min before use.
- Complete resuspension of the Auto-Mag® DTR by vortex

Sanger Sequencing Reaction Clean-up Process Overview



The workflow for Sanger sequencing reaction clean-up process is as follows:

1. Confirm the volume of Sanger sequencing reaction samples.
2. Add 10µl of Auto-Mag® DTR and the corresponding volume of 85% of Ethanol for DNA binding.
3. Separate beads from contaminants.

4. Wash beads/DNA twice with 85% of Ethanol to remove contaminants.
5. Elute purified DNA from beads.
6. Transfer eluate to new vessel and ready for sequencing.

The detailed procedure for Sanger sequencing reaction clean-up can be found in the following the 96 Well Format or 384 well format purification procedure in this manual.

Procedure for 96 Well Format

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

2. Add 10µl of Auto-Mag® DTR to each well contained sequencing reaction sample.

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

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

Sequencing Reaction Volume (µl)	85% Ethanol to be added (µl)
5	30
10	40
15	50
20	60
25	70
Note: Do not use denatured ethanol. Always prepare fresh 85% Ethanol. within 3 days of use and store tightly capped.	

4. Mix well the Auto-Mag® DTR reagent and sample by pipetting up and down 7-10 times.

5. Place the sample plate on the 96 magnetic separation device for 3-5 minutes or until the magnetic beads is completely cleared from solution.

6. With the sample plate still on the magnet, remove and discard all the liquid. Do not disturb the attracted beads.

7. Keep the sample plate on the magnet and add 100µl of 85% Ethanol to each well and wait 1-2 minute or until the magnetic beads are completely cleared from solution. Mixing is not necessary.

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

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

10. Keep the sample 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 reduce the yield.

11. Remove the sample plate from the magnet. Add 40µl of appropriate Elution Buffer (diH_2O or 0.1 mM EDTA) to each sample and mix by pipetting up and down 20 times.

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

13. Place the sample plate back on the magnet and wait 5 minutes or until the magnetic beads are completely cleared from Elution Buffer.

14. 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 transferring the elution.

Procedure for 384 Well Format

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

2. Add 5µl of Auto-Mag® DTR to each well contained sequencing reaction sample.

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

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

Sequencing Reaction Volume (µl)	85% Ethanol to be added (µl)
5	15
10	21
15	28
Note: Do not use denatured ethanol. Always prepare fresh 85% Ethanol. within 3 days of use and store tightly capped.	

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

5. Place the sample plate on the 384 magnetic separation device for 3-5 minutes or until the magnetic beads is completely cleared from solution.

6. With the sample plate still on the magnet, remove and discard all the liquid. Do not disturb the attracted beads.

7. Keep the sample plate on the magnet and add 30µl of 85% Ethanol to each well and wait 1-2 minute or until the magnetic beads is completely cleared from solution. Mixing is not necessary.

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

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

10. Keep the sample 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 reduce the yield.

11. Remove the sample plate from the magnet. Add 20µl of appropriate Elution Buffer (diH_2O or 0.1 mM EDTA) to each sample and mix by pipetting up and down 20 times.

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

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

cleared from Elution Buffer.

14. Transfer 15-18µl of the eluate (cleared supernatant) to a new plate to be loaded on a sequencer.

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

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Observation	Possible Causes	Comments
Dye 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 7-8, 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.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® DTR	S001-01 S001-02 S001-03 S001-04	5 ml 50 ml 250 ml 500 ml
Auto-Mag® Deionized Water	B228-01 B228-02 B228-03	50 ml 250 ml 500 ml

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