CleanDTR

Catalog Nos. CDTR-0005, CDTR-0050, CDTR-0500
Manual revision v2.02

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Introduction and Principle

CleanDTR is an efficient paramagnetic bead-based system, designed to remove unincorporated dye terminators from Sanger sequencing reaction. The CleanDTR process involves three simple steps including bind, wash and elute. While binding the sequencing product selectively to the magnetic particles, 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. The protocol can be adapted to your current liquid handling workstation (e.g. Beckman, Hamilton, Tecan, Caliper, Perkin Elmer, Agilent and Eppendorf) utilizing your current protocol as well as it can be performed manually.

Features:

- Long Phred 20 read lengths averaging over 800 bps
- Pass rates over 85% or higher
- Efficient elimination of sequencing reaction contaminants
- Reduce BigDye* usage, due to increased average signal strength

Applications

- 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

Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CDTR-0005	CleanDTR – 5 ml	500 *	4-8^{0}C DO NOT FREEZE
CDTR-0050	CleanDTR – 50 ml	5.000 *	
CDTR-0500	CleanDTR – 500 ml	50.000 *	

* Number of reactions is based on a typical 10 µL sequencing reaction volume in 96 well format.

Materials Supplied in the CleanDTR kit:
CleanDTR magnetic particle solution

Materials and Equipment to be supplied by User:

- 96-well PCR plate containing sequencing samples
- Magnetic separation device, recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50)
- Multichannel pipettor
- Multichannel Disposable Reservoirs
- 96-well microplate for elution
- 85% ethanol (freshly prepared from non-denatured alcohol)
- Elution Buffer (0,1 mM EDTA pH 8.0 or Di H2O)

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CleanDTR - 96-well Plate Protocol

1. Thoroughly shake the CleanDTR to fully resuspend the magnetic beads.

2. Add 10 µL CleanDTR to each well.

   Note: Use 10 µL CleanDTR regardless of the volume of the sequencing reaction.

3. Add 85% ethanol according to table below and mix the sample thoroughly by pipetting up and down 7-10 times.

   Note: Do not use denatured ethanol. Always prepare fresh 85% ethanol within 3 days of use and store tightly capped.

Reaction Volume (µL)	85% Ethanol (µL)
5	31
10	42
15	52
20	62

4. Place the plate on a magnetic separation device to magnetize the CleanDTR particles. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

5. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.

6. Add 100 µL 85% ethanol to each well. It is not necessary to resuspend the CleanDTR particles.

7. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.

9. Repeat Steps 6-8 for a second 85% ethanol wash step.

10. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanDTR particles. Remove any residue liquid with a pipettor.

    Note: It is important to completely remove all liquid from each well since it contains traces of unincorporated dyes and other contaminants.

11. Remove the plate from the magnetic separation device.

12. Add 40 µL Elution Buffer (0.1 mM EDTA or diH2O) to each well.

13. Pipet up and down 20 times to mix thoroughly.

14. Incubate at room temperature for 5 minutes.

15. Place the plate on a magnetic separation device to magnetize the CleanDTR particles. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

16. Transfer 30-35 µL cleared supernatant containing purified sequencing product to a new plate capable of being used in sequencer.

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CleanDTR - 384-well Plate Protocol

1. Thoroughly shake the CleanDTR to fully resuspend the magnetic beads.

2. Add 5 µL CleanDTR to each well.

   Note: Use 5 µL CleanDTR regardless of the volume of the sequencing reaction.

3. Add 85% ethanol according to table below and mix the sample thoroughly by pipetting up and down 7-10 times.

   Note: Do not use denatured ethanol. Always prepare fresh 85% ethanol within 3 days of use and store tightly capped.

Reaction volume (µL)	85% Ethanol (µL)
5	14,3
10	21,4
15	28,6

4. Place the plate on a magnetic separation device to magnetize the CleanDTR particles. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

5. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.

6. Add 30 µL 85% ethanol to each well. It is not necessary to resuspend the CleanDTR particles.

7. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.

9. Repeat Steps 6-8 for a second 85% ethanol wash step.

10. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanDTR particles. Remove any residue liquid with a pipettor.

    Note: It is important to completely remove all liquid from each well since it contains traces of unincorporated dyes and other contaminants.

11. Remove the plate from the magnetic separation device.

12. Add 15-20 µL Elution Buffer (0.1 mM EDTA or diH2O) to each well.

13. Pipet up and down 20 times to mix thoroughly.

14. Incubate at room temperature for 5 minutes.

15. Place the plate on a magnetic separation device to magnetize the CleanDTR particles. Incubate at room temperature until the CleanDTR particles are completely cleared from solution.

16. Transfer the cleared supernatant containing purified sequencing product to a new plate capable of being used in sequencer.

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Trouble Shooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Dye terminator remain in the eluted DNA and caused blobs	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 steps 6-9, mix beads to wash more effectively.
Low Signal	Ethanol concentration is not correct	Make sure to use correct volume of ethanol.
	Low ethanol concentration	Check the ethanol concentration, use fresh ethanol if necessary.
	Magnetic beads are lost during the process	Make sure not to remove any magnetic beads during aspiration.

Ordering Information

Contact your local distributor to order.

Product	Part Number
CleanDTR (5 mL)	CDTR-0005
CleanDTR (50 mL)	CDTR-0050
CleanDTR (500 mL)	CDTR-0500

Product	Part Number
Clean Magnet Plate 96-Well	CMAG-96-RN50

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