CleanDTR

Instructions for Use

V.5 - FEBRUARY 2025

For Research Use Only

REF **CDTR-0005, CDTR-0050, CDTR-0500**

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Contents

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. CleanXtract 96, Dynamic Devices LYNX™, Hamilton STAR™) utilizing your current protocol, or it can be performed manually.

Materials Provided

Kit Contents:

Product Number	Description	Number of Reactions
CDTR-0005	CleanDTR - 5 mL	500 x 10 µL reactions
CDTR-0050	CleanDTR - 50 mL	5000 x 10 µL reactions
CDTR-0500	CleanDTR - 500 mL	50000 x 10 µL reactions

Reagent Shipping, Storage and Handling

Shipping of CleanDTR should be done at room temperature (15-25 °C).

Do not freeze CleanDTR. After CleanDTR has been frozen, it is no longer suitable for use.

Component	Storage Temperature
CleanDTR	2-8 °C

Do not use CleanDTR after the expiration date on the label.

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

Make sure that the kit bottle is not damaged and that no liquid leaked from it. Do not use a kit that has been damaged.

Precautions

When working with chemicals, always follow your facility's procedures and universal precautions by using disposable gloves, safety glasses, a labcoat etc.

For all safety information, please consult the safety data sheet (SDS). Request your SDS via www.cleanna.com/resource-request.

CleanDTR	
No hazard pictogram	No precautionary statement(s) Prevention or Response

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of CleanDTR according to predetermined and validated protocols in the Quality Management System (QMS). Additionally, a quality check after production of each lot is performed to secure consistent product quality. CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

Materials and reagents to be supplied by user for CleanDTR protocols:

- 96- or 384-well PCR plate containing sequencing samples
- Magnetic separation device, recommended for 96 samples is the Clean Magnet Plate 96-Well RN50 (Part# CMAG-96-RN50)
- Multichannel pipettor
- Multichannel Disposable Reservoirs
- 96-well microplate for elution
- Vortex
- 85% ethanol (freshly prepared from non-denatured alcohol)
- Elution Buffer (0,1 mM EDTA pH 8.0, molecular biology grade water or formamide)

96-well Plate Protocol

Before Starting:

- Thoroughly resuspend the magnetic beads by vortexing.

Protocol:

1. **Vortex or shake CleanDTR vigorously to fully resuspend the magnetic particles.**
2. Add 10 µL of CleanDTR to each well.

⚠ **Note:** Add 10 µL of CleanDTR regardless **of the volume of the sequencing reaction.**

3. Add freshly prepared 85% ethanol according to the table below and mix thoroughly by pipetting or vortexing.

⚠ **Note:** 85% ethanol must be freshly made.

BigDye Reaction Volume (µL)	85% Ethanol Volume (µL)
5	31
10	42
15	52
20	62
25	73

4. Incubate at room temperature for 2-5 minutes.
5. Place the plate on a magnetic separation device for 2-5 minutes or until the particles are completely cleared from solution.
6. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.
7. With the plate on the magnetic separation device, add 100 µL of freshly prepared 85% ethanol to the magnetic bead pellet and incubate at room temperature for at least 30 seconds. Remove ethanol by pipetting.

⚠ **Note:** 85% ethanol must be freshly made.

8. Repeat step 7, for a total of 2 ethanol washes and ensure all ethanol has been removed.

9. Leave the plate on the magnetic separation device and let dry at room temperature for up to 5 minutes, or until the bead pellet is visibly dry but the surface still glossy.

⚠ **Note:** Do not over-dry the beads. Do not dry at higher temperature or under vacuum.

10. Remove the plate from the magnetic separation device. Resuspend dried beads with 40 µL of PCR-grade water, 0.1mM EDTA (pH 8.0), or formamide. Mix thoroughly by piptetting or vortexing. Ensure beads are no longer attached to the side of the well.
11. Incubate the resuspended beads at room temperature for 2-5 minutes.
12. Place the plate on the magnetic separation device for 2-5 minutes or until the sample appears clear.
13. Transfer 35 µL of the clear sample to a new plate. The samples are ready for loading on the sequencer. Alternatively, the samples can be stored at 4°C for up to 24 hours, or at -20°C for up to a month.

384-well Plate Protocol

Before Starting:

- Thoroughly resuspend the magnetic beads by vortexing.

Protocol:

1. Vortex or shake CleanDTR vigorously to fully resuspend the magnetic particles.
2. Add 5 µL of CleanDTR to each reaction of a 384-well PCR plate.

⚠ **Note:** Add 5 µL of CleanDTR regardless of the volume of the sequencing reaction.

3. Add freshly prepared 85% ethanol according to the table below and mix thoroughly by pipetting or vortexing.

⚠ **Note:** 85% ethanol must be freshly made.

BigDye Reaction Volume (µL)	85% Ethanol Volume (µL)
5	14.3
10	21.4

4. Incubate at room temperature for 2-5 minutes.
5. Place the plate on a magnetic separation device for 2-5 minutes or until the particles are completely cleared from solution.
6. Aspirate and discard the supernatant. Do not disturb the CleanDTR particles.
7. With the plate on the magnetic separation device, add 30 µL of freshly prepared 85% ethanol to the magnetic bead pellet and incubate at room temperature for at least 30 seconds. Remove ethanol by pipetting.

⚠ **Note:** 85% ethanol must be freshly made.

8. Repeat step 7, for a total of 2 ethanol washes and ensure all ethanol has been removed.
9. Leave the plate on the magnetic separation device and let dry at room temperature for up to 5 minutes, or until the bead pellet is visibly dry but surface still glossy.

⚠ **Note:** Do not over-dry the beads. Do not dry at higher temperature or under vacuum.

10. Remove the plate from the magnetic separation device. Resuspend dried beads with 15-30 µL of PCR-grade water, 0.1mM EDTA (pH 8.0), or formamide. Mix thoroughly by piptetting or vortexing. Ensure beads are no longer attached to the side of the well.

11. Incubate the resuspended beads at room temperature for 2-5 minutes.
12. Place the plate on the magnetic separation device for 2-5 minutes or until the sample appears clear.
13. Transfer the clear sample to a new plate. The samples are ready for loading on the sequencer. Alternatively, the samples can be stored at 4°C for up to 24 hours, or at -20°C for up to a month.

Troubleshooting 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	Suggestion
Dye terminator remain in the eluted DNA and caused blobs	Supernatant is not removed completely.	Make sure to grind samples completely.
	Too much BigDye®.	Use less BigDye® per reaction.
	Insufficient washing.	During steps 6-9, mix particles to wash more effectively.
Low Signal	Ethanol concentration is not correct.	Make sure to use correct volume of ethanol versus the reaction volume, as indicated in the table.
	Low ethanol concentration.	Check the ethanol concentration, use fresh ethanol if necessary.
	Magnetic particles are lost during the process.	Make sure not to remove any magnetic particles during aspiration.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

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 RN50	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
5	19/FEB/2025	Layout	According to new CleanNA corporate style.
		Protocol 96 well-plate	Added BigDye volume 25 µL to the table.
			Specified incubation times.
			Shortened bead drying step.
		Protocol 384 well-plate	Specified incubation times.
			Shortened bead drying step.
			Changed elution buffer volume.
		Total document	Linguistic clarifications.
4	01/OCT/2021	Total document	Linguistic clarifications.
		CleanDTR 96 well protocol, table	Changed x.0 µL to x µL.
		CleanDTR 96 well protocol and CleanDTR 384 well protocol	Replaced DiH20 with molecular biology grade water.
3	01/AUG/2020	Total revision	New lay-out.
		User manual information	General heading before contents added.

Contact

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