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HighPrep™ DTR

Dye Terminator Removal Clean Up

Catalog Nos. DT-70005, DT-70050, DT-70250, DT-70500
Manual Revision v1.06

- Magnetic beads based chemistry
- No centrifugation or filtration

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ DTR reagent is a paramagnetic bead-based system designed to remove unincorporated terminators from Sanger sequencing reactions. The protocol consists of a selective binding of DNA to the HighPrep™ DTR particles, follow with washing off nucleotides, primers and non-targeted amplicons, and finally elution of pure DNA to be directly used in downstream applications. HighPrep™ DTR is designed for both manual and fully automated purification of sequencing products. This protocol can be used for manual procedure as well as guideline for adapting it to automatic liquid handling workstations currently on the market. For availability of ready-to-run scripts please contact MagBio Genomics.

Process

HighPrep™ DTR uses a simple 3 steps procedure: Bind-Wash-Elute. HighPrep™ 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.

Product Specifications

Product Number	Description	Number of Reactions	Storage Conditions
DT-70005	HighPrep™ DTR - 5 mL	500	4-8°C **DO NOT FREEZE**
DT-70050	HighPrep™ DTR - 50 mL	5,000	
DT-70250	HighPrep™ DTR - 250 mL	25,000	
DT-70500	HighPrep™ DTR - 500 mL	50,000	

** Number of reactions is based on 10µL reaction volume. 10µl of HighPrep DTR is used regardless of the volume of the sequencing reaction.

Materials Supplied in the Kit

- HighPrep™ DTR paramagnetic beads solution
- Store at 4°C. DO NOT FREEZE. HighPrep™ DTR is stable for 12 months when stored at 4°C.
- Thoroughly shake the HighPrep™ DTR reagent to resuspend the beads before use.

Equipment and Reagents to Be Supplied by User:

- 85% ethanol ⚠ Prepare from absolute ethanol. Do not use denatured alcohol.
- Polypropylene reservoirs
- Elution buffer (0.1mM EDTA or Di H2O)

Magnet (Stand and Plate):

For 1.5mL tube format: MagBio MagStrip - Magnet Stand (1.5ml x 10)
MagBio Genomics, Inc., Cat# MBMS-10, www.magbiogenomics.com
For 96 well format: 96 well ring plate
For 384 well format: 384 magnet plate

Reaction Plate:

For 96 well format: 96 well cycling plate
For 384 well format: 384 well cycling plate

HighPrep™ DTR - 96 Well Format

1. **Bring HighPrep DTR to room temperature. Shake thoroughly the HighPrep DTR reagent to fully resuspend the magnetic beads.**

2. **Add 10 µl of HighPrep DTR reagent to each sample.**
   Use 10µl of HighPrep DTR regardless of the volume of the sequencing reaction.

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

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

⚠ * Do not use denatured alcohol. Prepare fresh from absolute ethanol.

4. **Mix well the HighPrep DTR reagent and sample by pipetting up and down 7-10 times.**

5. **Place the sample plate on the 96 magnetic separation device for 4-5 minutes or until the solution clears.** Beads will pull to the side of the well.

6. **With the plate still on the magnet, remove and discard the supernatant by pipetting.**

   ⚠ Do not disturb the attracted beads while aspirating the supernatant.

7. **Keep the sample plate on the magnet and add 100 µl of 85% ethanol to each well and wait 1-2 minutes or until the magnetic beads is fully resettled. Mixing is not necessary.**

8. **With the sample plate still on the magnet, remove and discard the supernatant by pipetting.**

9. **Repeat steps 7-8 for a total of two 85% ethanol washes.**

10. **Dry the beads by incubating for 10 minutes at room temperature with the plate still on the magnetic separation device.**

    ⚠ It is critical to completely remove all liquid from each well since it contains traces of excess fluorescent dye and other contaminants.

11. **Remove the sample plate from the magnet. Add 40 µl appropriate elution buffer (0.1mM EDTA or Di H_2O) to each well and pipet up and down 20 times to mix.**

12. **Incubate at room temperature for 5 minutes.**

13. **Place the sample plate back on the magnetic separation device and wait 5-7 minutes or until the magnetic beads clear from solution.**

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

HighPrep™ DTR - 384 Well Format

1. **Bring HighPrep DTR to room temperature. Shake thoroughly the HighPrep DTR reagent to fully resuspend the magnetic beads.**

2. **Add 5µl of HighPrep DTR reagent to each sample.**
   Use 5µl of HighPrep DTR regardless of the volume of the sequencing reaction.

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

Reaction Volume (µl)	85% Ethanol (µl)*
5	14.3
10	21.4
15	28.6

⚠ * Do not use denatured alcohol. Prepare fresh from absolute ethanol.

4. **Mix well the HighPrep DTR reagent and sample by pipetting up and down 7-10 times.**

5. **Place the sample plate on the 384 magnetic separation device for 3-4 minutes or until the solution clears.** Beads will pull to the side of the well.

6. **With the plate still on the magnet, remove and discard the supernatant by pipetting.**
   ⚠ Do not disturb the attracted beads while aspirating the supernatant.

7. **Keep the reaction plate on the magnet and add 30 µl of 85% ethanol to each well and wait 1-2 minutes or until the magnetic beads is fully resettled. Mixing is not necessary.**

8. **With the plate still on the magnet, remove and discard the ethanol by pipetting.**

9. **Repeat steps 7-8 for a total of two 85% ethanol washes.**

10. **Dry the beads by incubating for 10 minutes at room temperature with the plate still on the magnetic separation device.**
    ⚠ It is critical to completely remove all liquid from each well since it contains traces of excess fluorescent dye and other contaminants.

11. **Remove the reaction plate from the magnet. Add 15-20 µl appropriate elution buffer (0.1mM EDTA or Di H_2O) to each well and pipet up and down 20 times to mix.**

12. **Incubate at room temperature for 5 minutes.**

13. **Place the reaction plate back on the magnetic separation device and wait 5-7 minutes or until the magnetic beads clear from solution.**

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

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